ECOLOGIA BALKANICA
2020, Vol. 12, Issue 2 December 2020 pp. 141-151

CP43 and CP47 Proteins of Photosystem II (PSII) as Molecular Markers for Resolving Relationships between Closely Related Cyanobacteria

Dzhemal Moten[1*], Tsvetelina Batsalova[1], Rumen Mladenov[2], Balik Dzhambazov[1], Ivanka Teneva[2]

1 – University of Plovdiv „Paisii Hilendarski", Faculty of Biology, Department of Developmental Biology, 24 Tzar Assen Str., 4000 Plovdiv, BULGARIA
2 - University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Botany and Methods of BiologyTeaching, 24 TzarAssen Str., Plovdiv 4000, BULGARIA
[*]Corresponding author: moten@uni-plovdiv.bg

Abstract. Cyanobacteria are the most primitive photosynthetic organisms on the Earth. Their classification is traditionally based on morphological characters in both botanical and bacteriological systems. Due to the enormous diversity and the lack of clear diacritic morphological features between the closely related species, for resolving the evolutionary relationships and classification of Cyanobacteria during the last years is used a polyphasic approach including sequencing data. Although many molecular markers are improved, new suitable markers for resolving relationships within cyanobacteria at species and generic level are still needed. Our objective was to examine whether the sequences of the photosystem II proteins CP43 and CP47 are suitable markers for such purposes. Phylogenetic analyses based on the CP43 and CP47 amino acid sequences showed that most of the cyanobacterial species/strains belonging to different genera are clustered in separate clades supported by high bootstrap values. The comparison between the CP43 and CP47 trees, and the 16S phylogenetic trees showed that the CP43 and CP47 proteins are more suitable markers in resolving phylogenetic relationships within Cyanobacteria at generic and species level than the conserved 16S rRNA gene sequence. The correct taxonomic classification and identification of the cyanobacterial strains is very important for all studies related to the biological activity of cyanobacteria, their biotechnological application or in the management and monitoring of water.

Key words: Cyanobacteria, molecular marker CP43, CP47, phylogeny.

Introduction

Cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis. They can be found in almost all ecosystems on Earth including freshwater lakes, rivers, ponds, oceans, hot springs and deserts (Scanlan, 2001). Many cyanobacterial strains produce intracellular and extracellular metabolites with various biological activities (antibacterial, antifungal, antiviral, immunostimulation (Baldev et al., 2015), but they are also capable of extensive growth, resulting in bloom events and toxin production that can cause a significant threat to human and animal health (Carmichael, 1992).

Within prokaryotic groups, Cyanobacteria is one of the most morphologically diverse groups (Castenholz et al., 2001 & Shih et al., 2013). Their evolutionary relationships and classification are, at present, poorly understood (Castenholz et al., 2001 & Komarek et al., 2002). The taxonomy of Cyanobacteria has been debated vigorously and revised many times. One way to improve cyanobacterial taxonomy is a polyphasic approach including molecular, cytological, ecological, morphological, and physiological data (Rajaniemi et al., 2005). The morphological and cytological features are the basis for the conventional identification and taxonomy of cyanobacteria. But it is found that most of the cyanobacterial species change their morphology when grow at different ecological conditions. Therefore, the conventional methods are useless for identification of cyanobacteria at the species level. As a much more reliable technique for such identification is recommended the usage of molecular markers (Baldev et al., 2015).

Molecular phylogenies of cyanobacteria were mainly inferred with the 16S rDNA sequences. This molecular marker is generally conserved and provide many thousands of well-aligned informative sites. Furthermore, it is the best represented in the GenBank database, and phylogenies based on their sequences have so far been considered quite efficient for cyanobacterial phylogeny at the genus level (Hoffmann et al., 2005; Miller & Castenholz, 2001; Rajaniemi et al., 2005). The phylogenetic resolution of 16S rDNA, however, is limited, and often not sufficient to resolve relationships neither among very closely nor very distinctly related organisms (Johansen, 2005). Various protein coding sequences have also been used for inferring phylogenies within cyanobacteria (*rpoC1*, *rpoB*, *gyrB*, *rbcLX*, *cpcBA-IGS* and 16S-23S-*ITS)*(Sciuto et al., 2012; Seo & Yokota, 2003; Boyer et al., 2001; Nubel et al., 1997; Premanandh et al., 2006). The 5S ribosomal RNA, the outer membrane efflux protein (OMER), the Light-Repressed Protein (LRP) and the Psb27 protein have been recently proposed as new molecular markers (Teneva, 2019). The revolution in molecular phylogenetic approaches has had a profound effect on the description and classification of taxa (Garcia-Pichel et al., 2020).

Photosystem II (PSII) is the first component of the photosynthetic electron transfer chain located in the thylakoid membranes of cyanobacteria, algae and plants. Active cyanobacterial PSII consists of 17 transmembrane protein subunits, three peripheral proteins and about 80 cofactors such as chlorophylls, carotenoids and lipids (Guskov et al., 2009). The membrane-embedded core complex of PSII consists of the D1 and D2 reaction center (RC) subunits, the inner chlorophyll (Chl)-binding antenna proteins, CP43 and CP47, and a number of smaller polypeptides (Sánchez-Baracaldo & Cardona, 2020). CP47 and CP43 are encoded by the *psb*C and *psb*B genes in the genomic DNA of cyanobacteria. The main purpose of CP43 and CP47 is to deliver energy to the RC for driving electron transfer and, in the case of CP43, to help ligate the $CaMn_4$ cluster (Barber, 2006; Sánchez-Baracaldo & Cardona, 2020). CP43 and CP47 have 473 and 510 amino residues, respectively, and both of them have six transmembrane α-helices, which are separated by five extrinsic loop domains (Bricker & Frankel, 2002).

Taxonomy and classification have always been a challenge in the cyanobacteria. It is important to identify other reliable molecular markers for more precise cyanobacterial classification at the generic and subgeneric level. We decided to focus on the CP43 and CP47 proteins of Photosystem II, since so far, it is not exploited for phylogenetic analyses of Cyanobacteria. In this study, we investigated the phylogenetic relationships of cyanobacterial strains based on the CP43 and CP47 protein sequences, and compared the phylogenetic position of cyanobacterial strains with phylogenetic trees based on 16S rDNA.

Material and Methods

The CP43 and CP47 protein sequences used in this study were obtained from NCBI database. We chose to use amino acid sequences rather than nucleotide sequences because the latter are more strongly affected by saturation over long time scales (Li et al., 2014). The sequences were aligned to observe the phylogenetic relationship. Multiple sequence alignments of the proteins were created using ClustalW program of the phylogenetic software MEGA-7 (Kumar et al., 2016). Two data sets were constructed. The first one included 129 taxa (with *Escherichia coli str. K-12* as outgroup) and was based on CP47 sequence alignment. A second data set included 133 taxa (with *Arabidopsis thaliana* as outgroup) and was based on CP43 sequence alignment. The size of the analyzed CP47 amino acid sequences varied between 456 aa and 538 aa, and between 393 aa and 490 aa for CP43. The minimum evolution (ME), maximum parsimony (MP), maximum-likelihood (ML), and neighbor-joining (NJ) algorithms were used to construct phylogenetic trees, and the reliability of each branch was tested by 1,000 bootstrap replications. All positions containing gaps and missing data were removed from the dataset using the "complete deletion" option. For ME and NJ, the evolution distances were calculated using the Maximum Composite Likelihood method. For ML trees, the General Time Reversible (GTR) model with Corrected Invariable Sites (I), Gamma Distribution Shape Parameters (G), and Nearest-Neighbor-Interchange algorithm was used. In order to compare the topology of the taxa, the phylogenetic reconstruction was conducted also with 16S rDNA nucleotide sequences of the same strains used in the CP47 and CP43-phylogenetic trees. 16S rRNA gene sequences were aligned using the ClustalW multiple sequence alignment tools in Version 7.0 of MEGA phylogenetic software (Kumar et al., 2016). Trees based on the 16S rRNA gene were constructed by the same manner as described above. 16S rDNA nucleotide sequence from *Escherichia coli* str. K-12 was used to root the trees.

Results and Discussion

Our objective was to examine whether or not phylogenetic analysis based on CP47 and CP43 amino acid sequences supports the division Cyanobacteria. The phylogenetic trees based on different methods (Minimum Evolution (ME), Maximum Parsimony (MP), Maximum-Likelihood (ML), and Neighbor-Joining (NJ)) obtained in this study exhibited a high degree of similarity with minor topological differences. Here we represented only the ML trees.

CP43

In the present study, we have performed phylogenetic analyses based on the CP43 amino acid sequences. As can be seen in Fig. 1, cyanobacterial strains are grouped in eight larger clades (*Synechococcus* clade 1; *Candidatus Synechococcus* clade; *Planktothrix* clade; *Fischerela* clade; *Leptolyngbya* clade 1; *Nostoc* clade 1; *Nostoc* clade 2; *Pseudoanabaena* clade). Most members of the genus *Synechococcus* (23 strains) were grouped in two separate clades (Fig. 1, *Synechococcus* clade 1 (20 strains, 86% bootstrap supports), *Synechococcus* clade 2 (3 strains, 99% bootstrap supports)). The *Nostoc* strains also were clustered in two separate clades: *Nostoc* clade 1 (6 strains, 72% bootstrap support) and *Nostoc* clade 2 (4 strains, 65% bootstrap support, Fig. 1). All representatives of the other main clades were clustered in separate monophyletic clades: *Candidatus Synechococcus* (6 strains, 94% bootstrap support), *Planktothrix* (5 strains, 98% bootstrap support), *Pseudanabaena* (4 strains, 87% bootstrap support), and *Fischerella* (4 strains, 88% bootstrap support). The *Leptolyngbya* strains were clustered in three separate clades: *Leptolyngbya* clade 1 (4 strains, 69% bootstrap support, Fig. 1), *Leptolyngbya* clade 2 (3 strains, 54% bootstrap support, Fig. 1) and *Leptolyngbya* clade 3 (2 strains, 79% bootstrap support, Fig. 1). According to the phylogenetic analysis, the polyphyletic nature

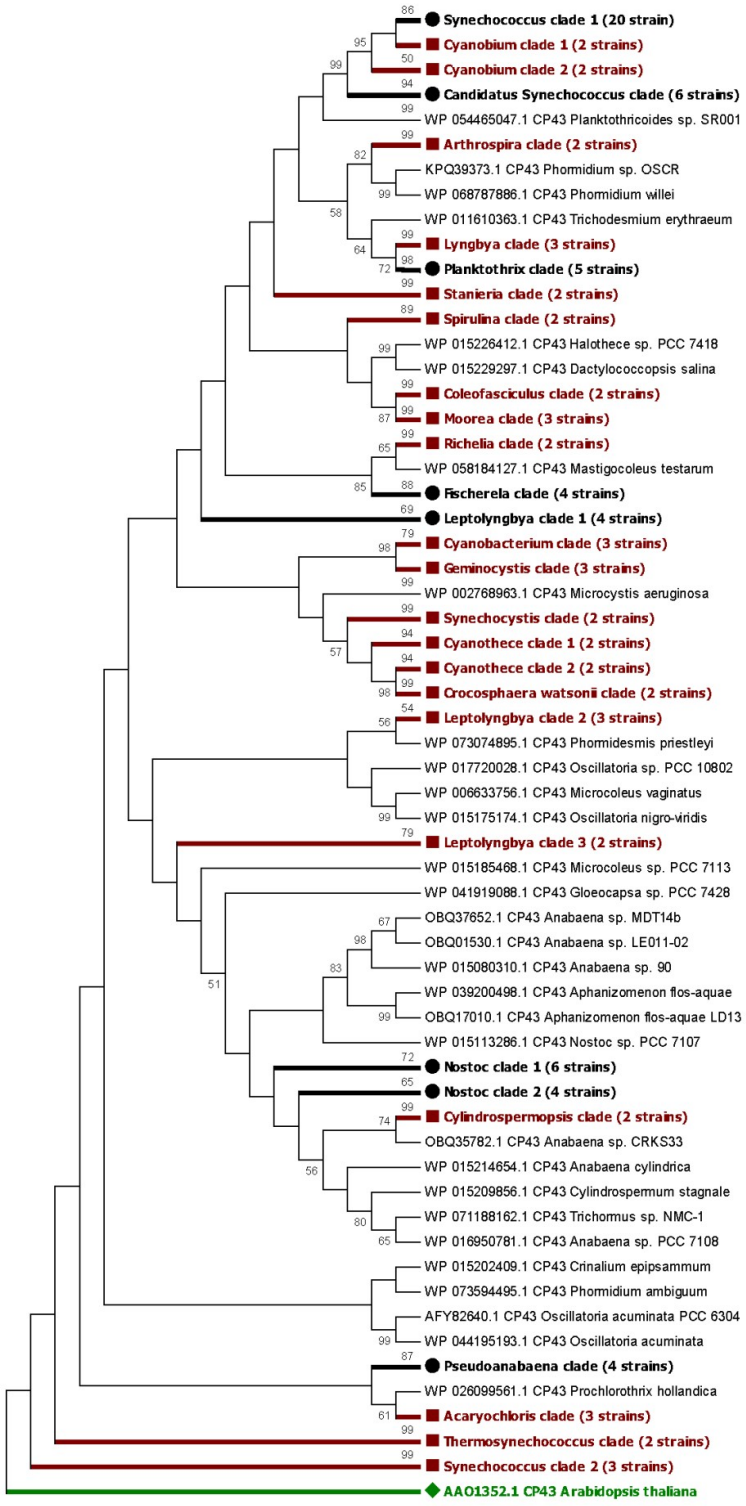

Fig. 1. Phylogenetic tree based on CP43 amino acid sequences from 133 cyanobacterial strains. The reconstruction has been performed by using ML analysis applying the GTR+I+G evolutionary model. The numbers above branches indicate the bootstrap support (>50%) from 1,000 replicates. *Arabidopsis thaliana* was used as an outgroup. ● - big monophyletic clades; ■ - small monophyletic clades.

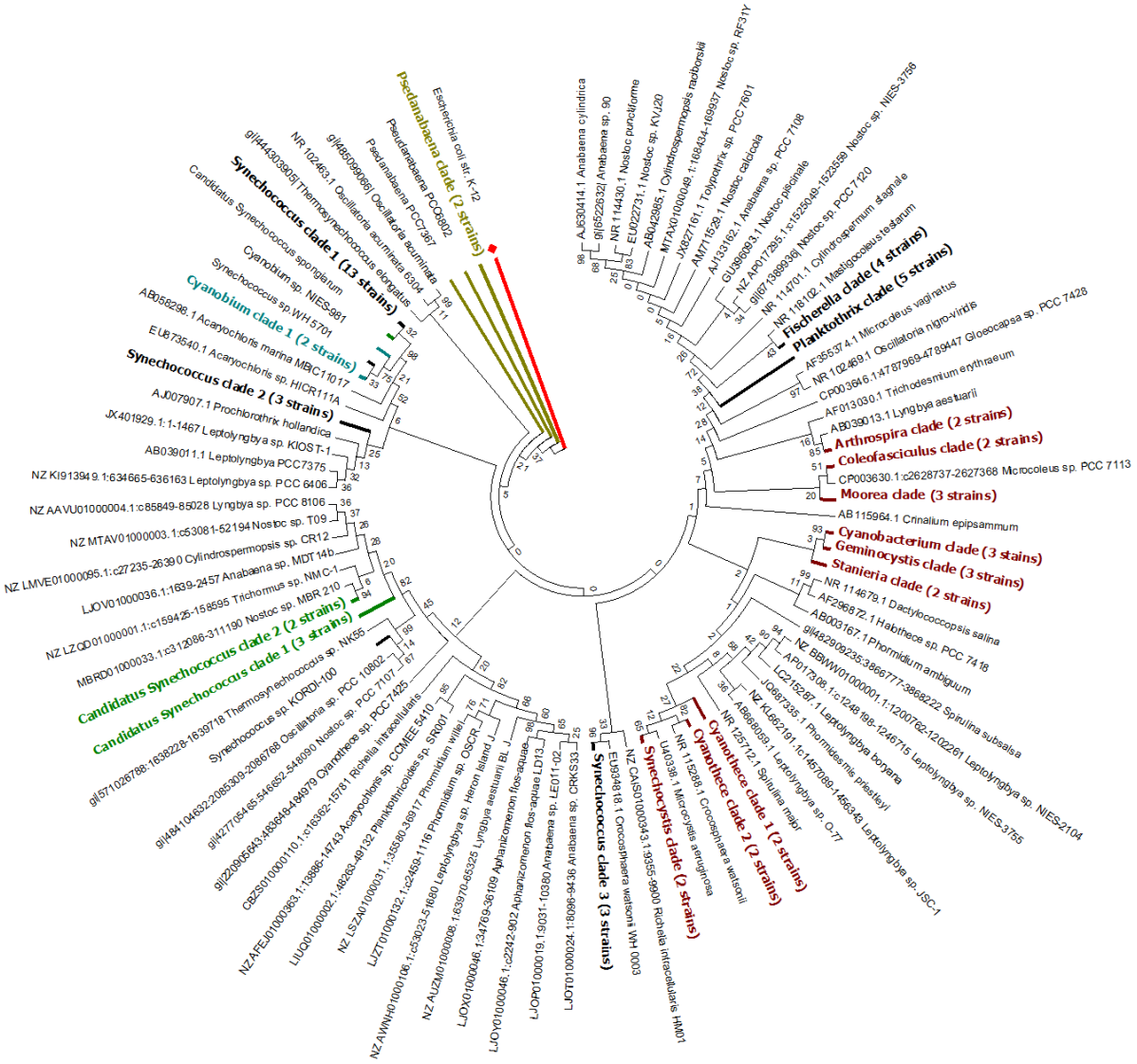

Fig. 2. Phylogenetic tree based on 16S rDNA sequences of 133 cyanobacterial strains. The tree was reconstructed by using ML analysis and the GTR+I+G evolutionary model. 16S rDNA sequence from *Escherichia coli* str. K-12 was used as an outgroup. The numbers near branches indicate bootstrap support from 1,000 replicates.

of order Oscillatoriales including genus *Oscillatoria, Microcoleus* and *Phormidium* was confirmed. Previous studies have also shown the polyphyletic nature of order Oscillatoriales based on the 16S rRNA gene sequences (Ishida et al., 1997; Marquardt & Palinska, 2007).

A number of smaller clades of cyanobacterial strains belonging to one genus, which are comprised of between 2 and 3 species/strains, are also observed in the tree shown in Fig. 1: *Cylindrospermopsis* clade (2 strains, 99% bootstrap support); *Moorea* clade (3 strains, 99% bootstrap support); *Arthrospira* clade (2 strains, 99% bootstrap support); *Spirulina* clade (2 strains, 89% bootstrap support); *Lyngbya* clade (3 strains, 99% bootstrap support); *Geminocystis* clade (3 strains, 99% bootstrap support); *Cyanobacterium* clade (3 strains, 79% bootstrap support); *Stanieria* clade (2 strains, 99% bootstrap support); *Richelia* clade (2

strains, 99% bootstrap support); *Synechocystis* clade (2 strains, 99% bootstrap support); *Cyanothece* clade 1 (2 strains, 94% bootstrap support); *Cyanothece* clade 2 (2 strains, 94% bootstrap support); *Thermosynechococcus* clade (2 strains, 99% bootstrap support); *Coleofasciculus clade* clade (2 strains, 99% bootstrap support); *Synechococcus* clade 2 (3 strains, 99% bootstrap support); *Cyanobium* clade 1 (2 strains, 50% bootstrap support); *Cyanobium* clade 2 (2 strains, 94% bootstrap support); *Acaryochloris* clade (3 strains, 99% bootstrap support); *Crocosphaera watsonii* clade (2 strains, 99% bootstrap support); *Leptolyngbya* clade 2 (3 strains, 54% bootstrap support); *Leptolyngbya* clade 3 (2 strains, 79% bootstrap support) (Fig. 1).

The phylogenetic trees based on the markers CP43 (Fig. 1) and 16S rDNA (Fig. 2) showed similar topologies, but differences in the positions of some strains were observed. Some of the species that showed monophyly with high bootstrap supports in the CP43 tree did not group together in the 16S tree. Intermixing of the members of genera *Synechococcus*, *Candidatus Synechococcus*, *Pseudanabaena*, *Nostoc*, *Thermosynechococcus, Cylindrospermopsis*, *Crocosphaera watsonii*, *Richelia* and *Spirulina* was observed in the phylogenetic tree based upon 16S rRNA, while in the phylogenetic tree constructed on CP43 they were grouped in separate monophyletic clades. The representatives of the genera *Fischerella, Planktothrix, Arthrospira, Coleofasciculus, Moorea, Cyanobacterium, Geminocystis, Stanieria,* and *Cyanothece* were grouped in separate monophyletic clades as in the phylogenetic tree based on CP43, but with low bootstrap supports (Fig. 2). The evolutionary relationships among different cyanobacterial taxa seen in this work are similar to those observed by Shih and colleagues (Shih et al., 2013). These results confirmed the usefulness of 16S rRNA gene as a valuable tool for identification of cyanobacteria up to order or genus level.

The phylogenetic trees based on the protein sequences and the 16S rRNA gene sequences created in this study showed that the CP43 is a suitable marker in resolving phylogenetic relationships within Cyanobacteria at generic and species level. The search for more stable molecular markers has become essential for understanding the phylogeny and taxonomy of cyanobacteria (Gribaldo & Brochier, 2009 & Makarova et al., 1999).

CP47

The phylogenetic tree based on CP47 amino acid sequences showed that most cyanobacterial species/strains belonging to one genus were clustered together and they were supported by high bootstrap values (Fig. 3). Eight distinct large monophyletic clades could be distinguished in the phylogenetic reconstruction, here named *Nostoc* clade 1, *Anabaena* clade 1, *Calothrix* clade, *Planktothrix* clade, *Pseudanabaena* clade, *Candidatus Synechococcus* clade, *Cyanobium* clade and *Synechococcus* clade 1. Representatives of the *Nostoc* (11 strains) were located in two separate clades (Fig. 3, *Nostoc* clade 1 (89% bootstrap supports), *Nostoc* clade 2 (53% bootstrap supports)). The *Anabaena* strains also were grouped in two separate clades: *Anabaena* clade 1 (5 strains, 91% bootstrap support) and *Anabaena* clade 2 (2 strains, 87% bootstrap support, Fig. 3). All representatives of the other main clades were clustered in separate monophyletic clades: *Calothrix* (4 strains, 97% bootstrap support), *Planktothrix* (6 strains, 100% bootstrap support), *Pseudanabaena* (4 strains, 67% bootstrap support), *Candidatus Synechococcus* (4 strains, 100% bootstrap support) and *Cyanobium* (4 strains, 78% bootstrap support). Most members of the genus *Synechococcus* were clustered in two separate clades: *Synechococcus* clade 1 (16 strains, 99% bootstrap support, Fig. 3) and *Synechococcus* clade 2 (3 strains, 98% bootstrap support, Fig. 3).

The other cyanobacterial species belonging to one genus were grouped in smaller separate clades: *Nodularia* clade (2 strains, 100% bootstrap support); *Cylindrospermopsis*

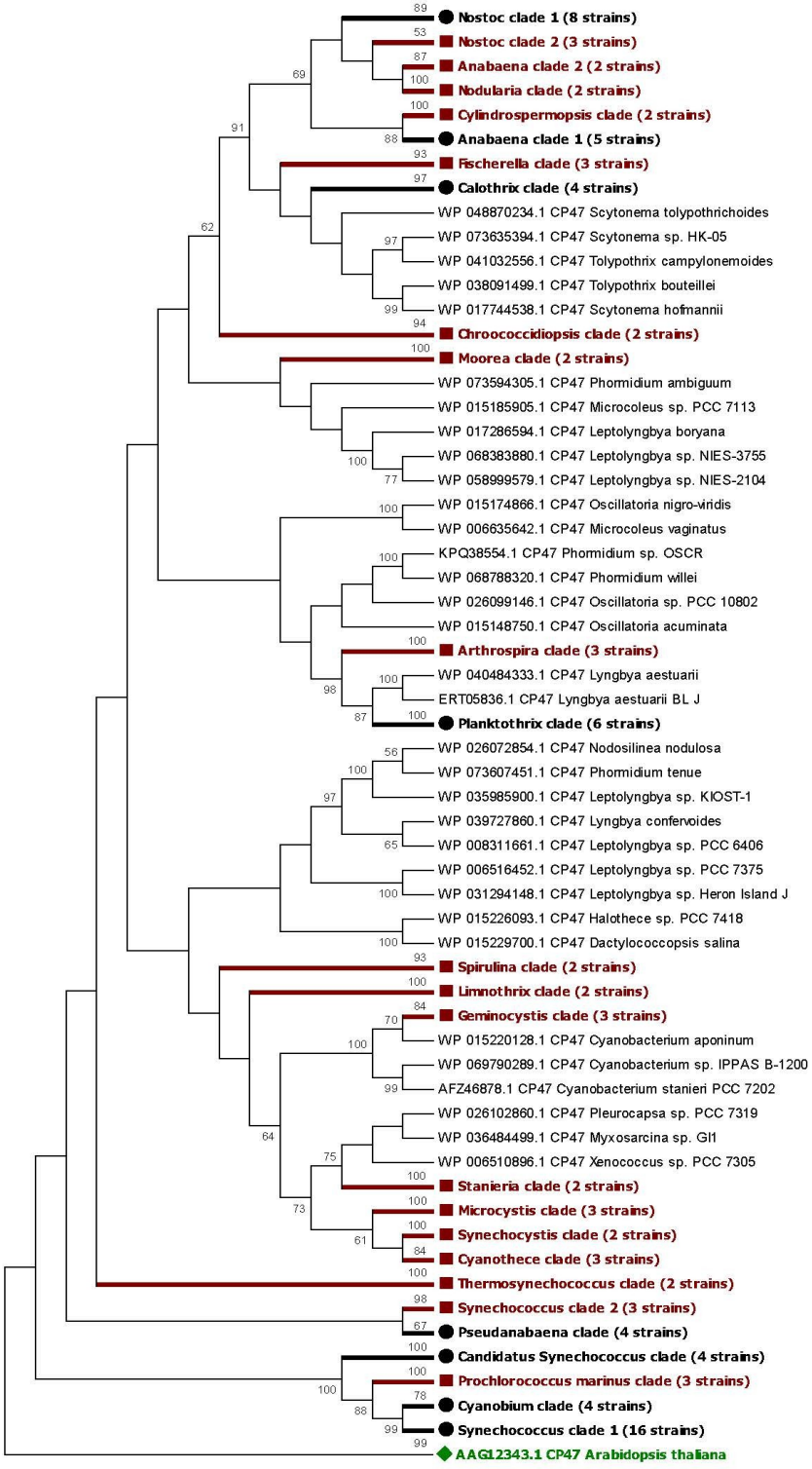

Fig. 3. Phylogenetic tree based on CP47 amino acid sequences from 129 cyanobacterial strains. The reconstruction has been performed by using ML analysis applying the GTR+I+G evolutionary model. The numbers above branches indicate the bootstrap support (>50%) from 1,000 replicates. *Arabidopsis thaliana* was used as an outgroup. ● - big monophyletic clades; ■ - small monophyletic clades.

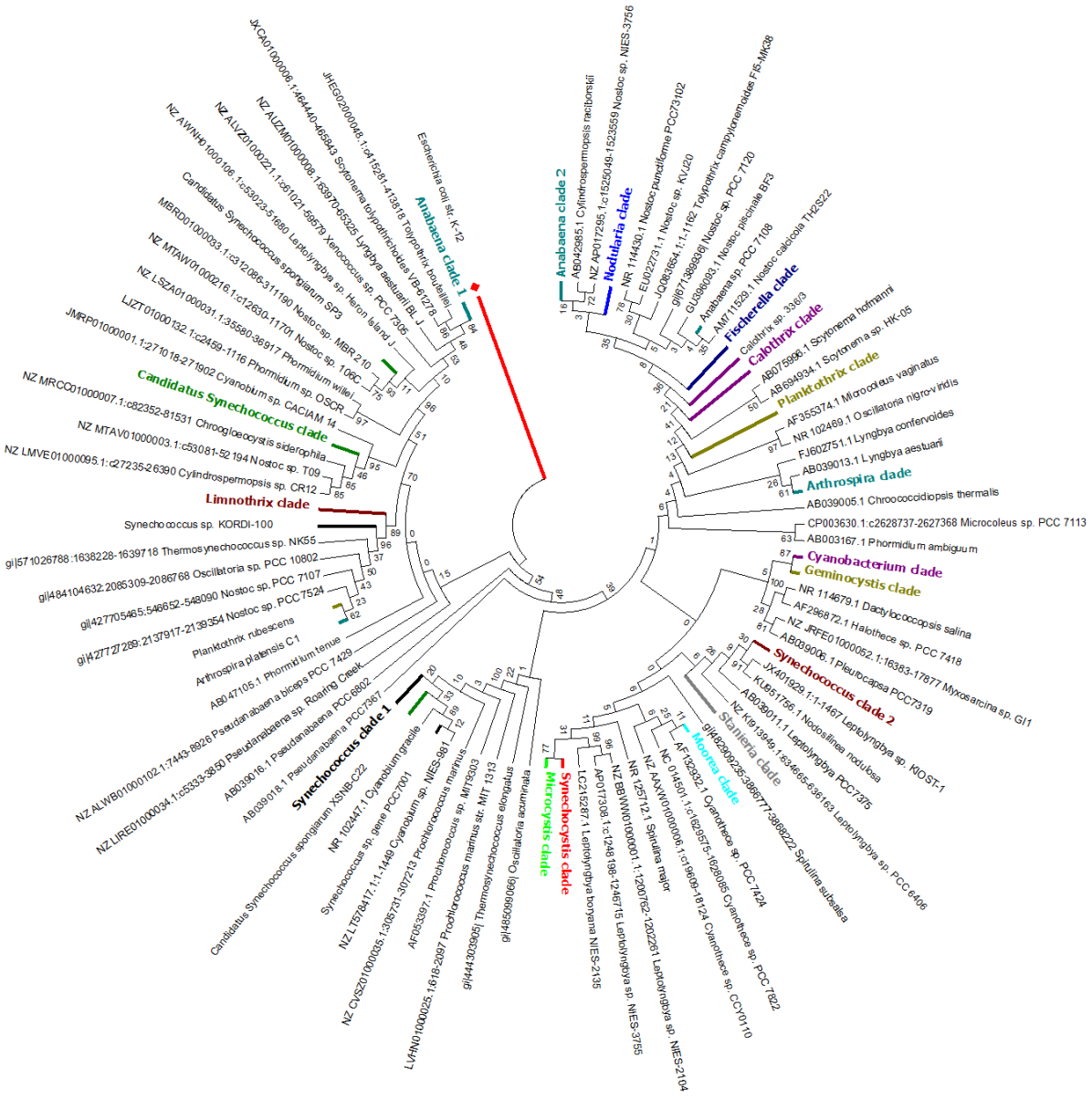

Fig. 4. Phylogenetic tree based on 16S rDNA sequences of 129 cyanobacterial strains. The tree was reconstructed by using ML analysis and the GTR+I+G evolutionary model. 16S rDNA sequence from *Escherichia coli* str. K-12 was used as an outgroup. The numbers near branches indicate bootstrap support from 1,000 replicates.

clade (2 strains, 100% bootstrap support); *Fischerella* clade (3 strains, 93% bootstrap support); *Chroococcidiopsis* clade (2 strains, 94% bootstrap support); *Moorea* clade (2 strains, 100% bootstrap support); *Arthrospira* clade (3 strains, 100% bootstrap support); *Spirulina* clade (2 strains, 93% bootstrap support); *Limnothrix* clade (2 strains, 100% bootstrap support); *Geminocystis* clade (3 strains, 84% bootstrap support); *Stanieria* clade (2 strains, 100% bootstrap support); *Microcystis* clade (3 strains, 100% bootstrap support); *Synechocystis* clade (2 strains, 100% bootstrap support); *Cyanothece* clade (3 strains, 84% bootstrap support); *Thermosynechococcus* clade (2 strains, 100% bootstrap support); *Prochlorococcus marinus* clade (3 strains, 100% bootstrap support);

Synechococcus clade 2 (3 strains, 98% bootstrap support); *Nostoc* clade 2 (3 strains, 53% bootstrap support); *Anabaena* clade 2 (3 strains, 87% bootstrap support); (Fig. 3). The examined cyanobacterial species formed a number of strongly supported clades in this tree.

For comparison, the 16S phylogenetic tree (Fig. 4) was constructed with 129 nucleotide sequences for the same cyanobacterial strains as in Fig. 3 using Mega 7 (Kumar et al., 2016). Comparing the topology of CP47 and 16S trees, it can be seen that the clades within the CP47 tree are clustered much better than within the 16S tree. Most of the species that showed monophyly with high bootstrap supports in the CP47 tree did not group together in the 16S tree. For example, *Nostoc, Pseudanabaena, Cyanobium, Anabaena, Calothrix, Synechococcus, Arthrospira, Candidatus Synechococcus* and *Planktothrix* (Fig. 4). Some of the other cyanobacterial species are also grouped in smaller separate clades as in the phylogenetic tree based on CP47, but with low bootstrap supports: *Nodularia* clade (2 strains, 85% bootstrap support); *Fischerella* clade (3 strains, 34% bootstrap support); *Geminocystis* clade (3 strains, 77% bootstrap support) (Fig. 4). Representatives of the *Cyanobacterium* (3 strains) were located in a separate clade within the 16S tree. This topology was supported by a bootstrap value of 64%.

The results presented herein strongly support CP43 and CP47 as markers of choice for cyanobacterial phylogenetic studies and emphasize the importance of using multiple molecular markers to prevent erroneous conclusions.

Conclusions

The taxonomic resolution offered by 16S rRNA genes is insufficient for discrimination of closely related organisms. The results obtained from this study have contributed greatly to the knowledge of cyanobacterial diversity. However, more phylogenetic studies are needed with other molecular markers to confirm the phylogenetic position of previously unidentified cyanobacterial isolates.

Acknowledgements

This research was financially supported by National program "Young scientists and Postdoctoral candidates" funded by the Bulgarian Ministry of Education and Science.

References

Baldev, E., MubarakAli, D., Dinesh Kumar, R. & Thajuddin, N. (2015). Molecular Phylogeny of Morphologically Diverse Cyanobacteria Based on Ribosomal Conserved Sequence. *Journal of Environmental Science and Technology*, 8, 188-197. doi: 10.3923/jest.2015.188.197.

Barber, J. (2006). Photosystem II: an enzyme of global significance. *Biochemical Society Transactions*, 34 (5), 619–631. doi: 10.1042/BST0340619.

Boyer, S.L., Flechtner, V.R. & J.R. Johansen. (2001). Is the 16S-23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. *Molecular Biology and Evolution,* 18, 1057-1069. doi: 10.1093/oxfordjournals.molbev.a003877

Bricker, T.M. & Frankel, L.K. (2002). The structure and function of CP47 and CP43 in Photosystem II. *Photosynthesis Research,* 72(2), 131-146. doi: 10.1023/a:1016128715865.

Carmichael, W.W. (1992). Cyanobacteria secondary metabolites-the cyanotoxins. *Journal of Applied Bacteriology*, 72, 445-459. doi: 10.1111/j.1365-2672.1992.tb01858.x.

Castenholz, R.W., Wilmotte, A., Herdman, M., Rippka, R., Waterbury, J.B., Iteman, I. & Hoffmann, L. (2001). *Phylum BX. Cyanobacteria. In Bergey's Manual® of Systematic Bacteriology: Volume One: The Archaea and the Deeply Branching and Phototrophic Bacteria.* pp. 473-599. New York, USA: Springer New York.

Garcia-Pichel, F., Zehr,, J.P., Bhattacharya, D. & Pakrasi, H.B. (2020). What's in a name? The case of cyanobacteria. *Journal of Phycology*, 56(1), 1-5. doi: 10.1111/jpy.12934.

Gribaldo, S. & Brochier, C. (2009). Phylogeny of prokaryotes: does it exist and why should we care? *Research in Microbiology*. 160 (7), 513-521. doi: 10.1016/j.resmic.2009.07.006.

Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A. & Saenger, W. (2009). Cyanobacterial photosystem II at 2.9-A resolution and the role of quinones, lipids, channels and chloride. *Nature Structural & Molecular Biology*, 16, 334-342. doi: 10.1038/nsmb.1559.

Hoffmann, L., Kaštovský, J. & Komárek, J. (2005). Proposal of cyanobacterial system, 2004. *Algological Studies,* 117(1), 95-115. doi: 10.1127/1864-1318/2005/0117-0095.

Komárek, J. & Anagnostidis, K. (2005). Cyanoprokaryota. 2. Teil: Oscillatoriales. In: Büdel, B., Gärtner, G., Krienitz,L. and Schagerl, M., Eds., Süßwasserflora von Mitteleuropa, Bd. Elsevier GmbH, München, 19(2), 1-759.

Ishida, K., Cao, Y., Hasegawa, M., Okada, N. & Hara, Y. (1997). The origin of chlorarachniophyte plastids, as inferred from phylogenetic comparisons of amino acid sequences of EF-Tu. *Journal of Molecular Evolution*, 45, 682-687. doi: 10.1007/PL00006272.

Johansen, J.C., Casamatta, D.A. (2005). Recognizing cyanobacterial diversity through adoption of a new species paradigm. *Algological Studies*, 117, 171–193. doi: 10.1127/1864-1318/2005/0117-0071.

Komarek, P., Kleisner, I., Konopkova, M. & Komarkova, I. (2002). Biodistribution of low-molecular-weight proteins and technetium (99mTc) labeled peptides in an animal model of inflammation. *Ceska a Slovenska farmacie*, 51, 252-256.

Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution,* 33, 1870-1874.

Li, B., Lopes, J.S., Foster, P.G., Embley, T.M. & Cox, C.J. (2014). Compositional Biases among Synonymous Substitutions Cause Conflict between Gene and Protein Trees for Plastid Origins. *Molecular Biology and Evolution*, 31, 1697-1709. doi: 10.1093/molbev/msu105.

Makarova, K.S., Aravind, L., Galperin, M.Y., Grishin, N.V., Tatusov, R.L., Wolf, Y.I. & Koonin, E.V. (1999). Comparative genomics of the Archaea (Euryarchaeota): evolution of conserved protein families, the stable core, and the variable shell. *Genome Research,* 9, 608-628. doi: 10.1101/gr.9.7.608.

Marquardt, J. & Palinska, K.A. (2007). Genotypic and phenotypic diversity of cyanobacteria assigned to the genus *Phormidium (Oscillatoriales)* from different habitats and geographical sites. *Archives of Microbiology,* 187, 397-413. doi: 10.1007/s00203-006-0204-7.

Miller, S.R. & Castenholz, R.W. (2001). Ecological physiology of Synechococcus sp. strain SH-94-5, a naturally occurring cyanobacterium deficient in nitrate assimilation. *Applied and Environmental Microbiology*, 67, 3002-3009. doi: 10.1128/AEM.67.7.3002-3009.2001.

Nubel, U., Garcia-Pichel, F. & Muyzer, G. (1997). PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and Environmental Microbiology*, 63, 3327-3332. doi: 10.1128/AEM.63.8.3327-3332.1997.

Premanandh, J., Priya, B., Teneva, I., Dzhambazov, B., Prabaharan, D. & L. Uma. 2006. Molecular characterization

of marine Cyanobacteria from the Indian subcontinent deduced from sequence analysis of the phycocyanin operon (cpcb-IGS-cpcA) and 16S-23S ITS region. *Journal of Microbiology,* 44, 607-616.

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, 11-26. doi: 10.1099/ijs.0.63276-0.

Sánchez-Baracaldo, P., & Cardona, T. (2020). On the origin of oxygenic photosynthesis and Cyanobacteria. *New Phytologist*, 225, 1440-1446. doi: 10.1111/nph.16249.

Scanlan, D. 2001. Cyanobacteria: ecology, niche adaptation and genomics. *Microbiology Today*, 28, 128-130.

Sciuto, K., Andreoli, C., Rascio, N., La Rocca, N. & Moro, I. (2012). Polyphasic approach and typification of selected *Phormidium* strains (Cyanobacteria). *Cladistics*, 28, 357-374. doi: 10.1111/j.1096-0031.2011.00386.x.

Seo, P.S. & Yokota, A. (2003). The phylogenetic relationships of cyanobacteria inferred from 16S rRNA, gyrB, rpoC1 and rpoD1 gene sequences. *The Journal of General and Applied Microbiology,* 49, 191-203. doi: 10.2323/jgam.49.191.

Shih, P.M., Wu, D., Latifi, A., Axen, S.D., Fewer, D.P., Talla, E., Calteau, A., Cai, F., Tandeau de Marsac, N., Rippka, R., Herdman, M., Sivonen, K., Coursin, T., Laurent, T., Goodwin, L., Nolan, M., Davenport, K.W., Han, C.S., Rubin, E.M., Eisen, J.A., Woyke, T., Gugger, M. & Kerfeld, C.A. (2013). Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. *Proceedings of the Nacional Academy of Sciences of the Unated States of America*, 110, 1053-1058. doi: 10.1073/pnas.1217107110.

Teneva, I., Belkinova, D, Moten, D., Basheva, D. & Dzhambazov, B. (2019). In search of new molecular markers for taxonomic classification of cyanobacteria. *18th International multidisciplinary scientific conference SGEM 2018 Conference proceedings, Bulgaria*, 19(5.2), 461-468. doi: 10.5593/sgem2019/5.2/S20.057.

Received: 11.09.2020
Accepted: 14.12.2020