Phytotaxa 192 (1): 001–012
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.192.1.1

Polyphasic characterization of four species of *Pseudanabaena* (Oscillatoriales, Cyanobacteria) from China and insights into polyphyletic divergence within the *Pseudanabaena* genus

GONGLIANG YU[1,2,§], MENGLING ZHU[3,§], YOUXIN CHEN[1,4], QIANQIAN PAN[1], WENBO CHAI[1] & RENHUI LI[1*]

[1] *Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, PR China.*
[2] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan.*
[3] *Key Laboratory of Ecological Impacts of Hydraulic-Projects and Restoration of Aquatic Ecosystem of Ministry of Water Resources, Institute of Hydroecology, Ministry of Water Resources and Chinese Academy of Sciences*
[4] *University of Chinese Academy of Sciences, Beijing 100049, P.R. China.*
[§] *Equally contributed to this work.*
[*]*Correspondence author: E-mail reli@ihb.ac.cn*
Tel. (+86) 27 68780067; Fax (+86) 27 68780123

Abstract

Current cyanobacterial taxonomic studies are mainly performed through polyphasic characterization at the genus and/or species levels. In this study, eleven *Pseudanabaena* strains isolated in China were taxonomically and phylogenetically evaluated by investigating several characteristics, including morphological features, cellular ultrastructures, pigment composition, and 16S rRNA gene sequences. The strains were identified as four morphospecies, namely, *Pseudanabaena mucicola*, *P. galeata*, *P. limnetica*, and *P. minima*. The cellular ultrastructures of these species showed that peripheral thylakoids with 4–8 parallel layers were parietally distributed. Phylogenetic results based on 16S rRNA gene sequences indicated that most of the *Pseudanabaena* strains used in this study, including the eleven from China and three *Pseudanabaena catenata* strains from GenBank, gathered into one large cluster that represents the core of the genus *Pseudanabaena.* Several *Pseudanabaena* species/strains that represent different extreme environments were phylogenetically located outside the main stream cluster and should be removed under the genus *Pseudanabaena* on the basis of the role of the main stream species. *Limnothrix* strains were divided into two clades, and the taxonomic solution for these clades was discussed. This study is the first to report on the morphology and phylogeny of *Pseudanabaena* species in China, providing new insights into the taxonomy of the genera *Pseudanabaena* and *Limnothrix*.

Key words: China, cyanobacteria, *Limnothrix*, morphology, phylogeny, *Pseudanabaena*, taxonomy

Introduction

Comprehensive studies on water-bloom-forming cyanobacteria have been magnified because of the harmful effects of toxin production and the unpleasant taste and odor caused by these cyanobacteria (Peterson *et al*. 1995, DeVries *et al*. 2012). It is well known for those bloom-forming cyanobacteria, which have relatively large colony/filament sizes and can float on water surface through their gas vesicles. However, tiny cyanobacteria species, including those related to water blooms, have been overlooked because of difficulties in observation brought about by their relatively small cell/filament sizes. Occasionally, these cyanobacteria dominate phytoplankton biomass (Romo and Miracle 1994, Sthapit *et al*. 2008), however, they were usually described with few characters or only recorded as species names probably because of poor understanding of their traits (Mischke and Nixdorf 2003, Villena and Romo 2003). These cyanobacteria are important in ecosystem dynamics and primary productivity.

Anagnostidis and Komárek (1988: 380) described the genus *Pseudanabaena* Lauterborn (1915: 437) as a group of tiny cyanobacteria. With *Pseudanabaena catenata* Lauterborn (1915: 437) (Chang 1988) as the type species, *Pseudanabaena* is characterized by the presence of filaments with conspicuous constrictions at cross-walls consisting of cylindrical cells. Some species that contain both phycocyanin (PC) and phycoerythrin (PE) display complementary chromatic adaptation that allows the regulation of PC/PE ratio to maximize the absorption of available light (Kehoe and Gutu 2006, Stomp *et al*. 2004, 2008).

Accepted by Saúl Blanco Lanza: 14 Nov. 2014; published: 7 Jan. 2015

As described above, the *Limnothrix* strains shown in the phylogenetic tree were mainly divided into two clades (Fig. 4). The first clade included three strains of *Limnothrix redekei* (NIVA–227, CCAP1443/1, and CCAP 1459/29), whereas the second clade included strains of *L. redekei* (2LT25S01 and 165c) and *L. planktonica* CHAB753. The first *Limnothrix* clade was fully embedded in Cluster I with the type species and most *Pseudanabaena* strains. The *L. redekei* strains in this clade should be transferred under *Pseudanabaena* on the basis of the real *Pseudanabaena* cluster described above and later time of establishment of *L. redekei.* The second *Limnothrix* clade was positioned outside the *Pseudanabaena* cluster, suggesting that these *Limnothrix* strains should remain under *Limnothrix.* Such a result partially agreed with the treatment by Whitton (2011), who used *Pseudanabaena redeckei* (Goor 1918: 258) B.A.Whitton (2011: 110) as the synonym of *Limnothrix redekei*. Future studies on the taxonomic ambiguity of *Limnothrix* are needed. Investigation of other genes (e.g. *rbcLX*, *rpoC1*, *cpcBA* etc.) might be helpful.

In conclusion, the polyphasic characterization of *Pseudanabaena* strains isolated in China allowed us to establish the main stream species of *Pseudanabaena*. The phylogenetic relationship shown in this study by adding *Pseudanabaena* strains from Chinese waters provided new insights into the taxonomic revision of both *Pseudanabaena* and *Limnothrix*.

Aknowlegements

This research was supported by National Natural Science Foundation of China (No. 30970185), by JSPS RONPAKU (Dissertation PhD) Program, and also by Major Science and Technology Program for Water Pollution Control and Treatment (No. 2012ZX07101-002-001 and No. 2012ZX07105-004). We appreciate efforts and the valuable comments from reviewers and editors.

References

Acinas, S.G., Haverkamp, T.H.A., Huisman, J. & Stal, L.J. (2009) Phenotypic and genetic diversification of *Pseudanabaena* spp. (cyanobacteria). *ISME Journal* 3: 31–46. http://dx.doi.org/10.1038/ismej.2008.78

An, G.S. (1992) Validation of new species names of blue-green algae Cyanophyta from Korea. *Fragmenta Floristica et Geobotanica* 37(2): 407–416.

Anagnostidis, K. (2001) Nomenclatural changes in cyanoprokaryotic order Oscillatoriales. *Preslia, Praha* 73: 359–375.

Anagnostidis, K. & Komárek, J. (1988) Modern approach to the classification system of cyanophytes 3. Oscillatoriales. *Algological Studies* 50–53: 327–472.

Böcher, T.W. (1949) Studies on the sapropelic flora of the lake Flyndersø with special reference to the Oscillatoriaceae. *Kongelige Danske Videnskabernes Selskab Biologiske Meddelelser* 21: 1–46.

Casamatta, D.A., Johansen, J.R., Vis, M.L. & Broadwater, S.T. (2005) Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria). *Journal of Phycology* 41: 421–438. http://dx.doi.org/10.1111/j.1529-8817.2005.04062.x

Chang, T. (1988) Morphological remarks on *Pseudanabaena mucicola* (Huber-Pestalozzi et Naumann) (Bourrelly) Chang. *Algological Studies* 80: 1–4.

Desikachary, T.V. 1959. *Cyanophyta*. Indian Council of Agriculture Research, New Delhi, pp. 81–132.

DeVries, S.L., Liu, W., Wan, N., Zhang, P. & Li, X. (2012) Biodegradation of MIB, geosmin and microcystin-LR in sand columns containing Taihu lake sediment. *Water Science and Technology*: *Water Supply* 12(5): 691–698. http://dx.doi.org/10.2166/ws.2012.043

Dignum, M., Hoogveld, H.L., Matthijs, H.C.P., Laanbroek, H.J. & Pel, R. (2004) Detecting the phosphate status of phytoplankton by enzyme-labelled fluorescence and flow cytometry. *FEMS Microbiology Ecology* 48: 29–38. http://dx.doi.org/10.1016/j.femsec.2003.12.007

Fritsch, F.E. (1945) *The Structure and Reproduction of the Algae.* Cambridge University Press, Cambridge, 939 pp.

Geitler, L. (1932) Cyanophyceae. *In:* Rabenhorst, L. (Ed.) *Kryptogamen Flora von Deutschland, Osterreich und der Schweiz 14*. Akademische Verlagsgesellschaft, Leipzig, pp. 130–159.

Gomont, M. (1892) Monographie des Oscillariées (Nostocacées homocystées). [Séries 7] *Annales des Sciences Naturelles, Botanique* 15: 263–368.

Goor, A.C.J. van (1918) Zur Kenntnis der Oscillatoriaceen. *Reçueil des Travaux Botaniques Néerlandais* 15: 255–262.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systems Biology* 52: 696–704.
http://dx.doi.org/10.1080/10635150390235520

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Huber-Pestalozzi, G. & Naumann, E. (1929) *Phormidium mucicola* Naumann et Huber, ein Epibiont in der Gallerte pflanzlicher und tierischer Planktonorganismen. *Berichte der Deutschen Botanischen Gesellschaft* 47(1): 67–76.
http://dx.doi.org/10.1111/j.1438-8677.1929.tb01573.x

Kasai, F., Kawachi, M., Erata, M., Mori, F., Yumoto, K., Sato, M. & Ishimoto, M. (2009) NIES-Collection, List of Strains, 8th edn. *The Japanese Journal of Phycology* 57(1): 216–217.

Kehoe, D.M. & Gutu, A. (2006) Responding to color: the regulation of complementary chromatic adaptation. *Annual Review of Plant Biology* 57: 127–150.
http://dx.doi.org/10.1146/annurev.arplant.57.032905.105215

Kim, S.G., Rhee, S.K., Ahn, C.Y., Ko, S.R., Choi, G.G., Bae, J.W., Park, Y.H. & Oh, H.M. (2006) Determination of cyanobacterial diversity during algal blooms in Daechung Reservoir, Korea, on the basis of cpcBA intergenic spacer region analysis. *Applied and Environmental Microbiology* 72(5): 3252–3258.
http://dx.doi.org/10.1128/AEM.72.5.3252-3258.2006

Kling, H.J. & Watson, S. (2003) A new planktic species of *Pseudanabaena* (Cyanoprokaryota, Oscillatoriales) from North American large lakes. *Hydrobiologia* 502: 383–388.
http://dx.doi.org/ 10.1023/B:HYDR.0000004295.11437.df

Kling, H.J., Laughinghouse IV, H.D., Šmarda, J., KomáreK, J., Acreman, J., Bruun, K., Watson, S.B. & Chen, F. (2012) A new red colonial *Pseudanabaena* (Cyanoprokaryota, Oscillatoriales) from North American large lakes. *Fottea Olomouc* 12(2): 327–339.

Komárek, J. (1974) Taxonomische Bemerkungen zu einigen Arten der Mikroflora der Teiche in Böhmen. *Acta Scientiarum Naturalium Musei Bohemici Meridionalis Ceské Budejovice* 14: 161–190.

Komárek, J. (2003) Planktic oscillatorialean cyanoprokaryotes (short review according to combined phenotype and molecular aspects). *Hydrobiologia* 502: 367–382.
http://dx.doi.org/10.1023/B:HYDR.0000004294.17755.fe

Komárek, J. & Anagnostidis, K. (2005) *Cyanoprokaryota 2. Teil: Oscillatoriales*. Elsevier GmbH, Heifelberg, 70 pp.

Kützing, F.T. (1833) Beitrag zur Kenntniss über die Entstehung und Metamorphose der niedern vegetabilischen Organismen, nebst einer systematischen Zusammenstellung der hieher gehörigen niedern Algenformen. *Linnaea* 8: 335–384.

Kützing, F.T. (1846) *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. I.* Gedruckt auf kosten des verfassers (in commission bei W. Köhne), Nordhausen, 54 pp.
http://dx.doi.org/10.5962/bhl.title.4971

Lauterborn, R. (1915) Die sapropelische Lebewelt. Ein Beitrag zur Biologie des Faulschlammes natürlicher Gewässer. *Verhandlungen des Naturhistorisch– Medizinischen Vereins zu Heidelberg, Neue Folge* 13: 395–481.

Lemmermann, E. (1900) Beiträge zur Kenntnis der Planktonalgen. X. Diagnosen neuer Schwebalgen. *Berichte der deutsche botanischen Gesellschaft* 18: 306–310.

Lin, S., Wu, Z.X., Yu, G.L., Zhu, M.L., Yu, B.S. & Li, R.H. (2010) Genetic diversity and molecular phylogeny of *Planktothrix* (Oscillatoriales, cyanobacteria) strains from China. *Harmful Algae* 9(1): 87–97.
http://dx.doi.org/10.1016/j.hal.2009.08.004

Meffert, M.E. (1988) *Limnothrix* Meffert nov. gen. The unsheathed planktic cyanophycean filaments with polar and central gas vacuoles. *Algological Studies* 50–53: 269–276.

Mischke, U. & Nixdorf, B. (2003) Equilibrium phase conditions in shallow German lakes: How Cyanoprokaryota species establish a steady state phase in late summer. *Hydrobiologia* 502: 123–132.
http://dx.doi.org/10.1007/978-94-017-2666-5_11

Mitchell, B.G. (1990) Algorithms for determining the absorption coefficient of aquatic particulates using the quantitative filter technique (QFT). *In*: Spinrad, R.W. (Ed.) *Ocean Optics X, Proceedings-Society of Photo-Optical Instrumentation Engineers* 1302: 137–148.
http://dx.doi.org/10.1117/12.21440

Neilan, B.A., Jacobs, D. & Goodman, A.E. (1995) Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. *Applied Environmental Microbiology* 61: 3327–3332.

Neilan, B.A., Jacobs, D.T., Del, Dot., Blackall, L.L., Hawkins, P.R., Cox, P.T. & Goodman, A.E. (1997) rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic Bacteriolology* 47: 693–697.
http://dx.doi.org/10.1099/00207713-47-3-693

Peterson, H.G., Hrudey, S.E., Cantin, I.A., Perley, T.R. & Kenefick, S.L. (1995) Physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by *Aphanizomenon flos-aquae* after exposure to water treatment chemicals. *Water*

Research 29(6): 1515–1523.
http://dx.doi.org/10.1016/0043-1354(94)00300-V

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14(9): 817–818.
http://dx.doi.org/10.1093/bioinformatics/14.9.817

Rippka, R. (1988) Isolation and purification of cyanobacteria. *Methods in Enzymology* 167: 3–27.
http://dx.doi.org/10.1016/0076-6879(88)67004-2

Romo, S. & Miracle, M.R. (1994) Population dynamics and ecology of subdominant phytoplankton speciesin a shallow hypertrophic lake (Albufera of Valencia, Spain). *Hydrobiologia* 273: 31–56.
http://dx.doi.org/10.1007/BF00126767

Schwabe, G.H. (1964) Grundprobleme der Cyanophytentaxonomie. *Gewässer und Abwässer* 36: 7–39.

Shao, J.H., Xu, Y., Wang, Z.J., Jiang, Y.G., Yu, G.L., Peng, X. & Li, R.H. (2011) Elucidating the toxicity targets of β-ionone on photosynthetic system of *Microcystis aeruginosa* NIES-843 (Cyanobacteria). *Aquatic Toxicology* 104: 48–55.
http://dx.doi.org/10.1016/j.aquatox.2011.03.014

Stackebrandt, E. & Ebers, J. (2006) Taxonomic parameters revisited: tarnished gold standards. *Microbiology Today* 33: 152–155.

Stackebrandt, E. & Goebel, B.M. (1994) A place for DNA–DNA reassociation and 16S ribosomal–RNA sequence–analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology* 44: 846–849.
http://dx.doi.org/10.1099/00207713-44-4-846

Starmach, K. & Sieminska, J. (1966) *Flora słodkowodna Polski, Volume 2.* Polska Akademia Nauk Instytut Botaniki, Warsaw, Kraków, pp. 445–452.

Sthapit, E., Ochs, C.A. & Zimba, P.V. (2008) Spatial and temporal variation in phytoplankton community structure in a southeastern U.S. reservoir determined by HPLC and light microscopy. *Hydrobiologia* 600: 215–228.
http://dx.doi.org/10.1007/s10750-007-9234-7

Stomp, M., Huisman, J., de Jongh, F., Veraart, A.J., Gerla, D. & Rijkeboer, M. (2004) Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature* 432: 104–107.
http://dx.doi.org/10.1038/nature03044

Stomp, M., van Dijk, M.A., van Overzee, H.M.J., Wortel, M., Sigon, C.A.M. & Egas, M. (2008) The time scale of phenotypic plasticity, and its impact on competition in fluctuating environments. *American Naturalist* 172(5): 169–185.
http://dx.doi.org/10.1086/591680

Suda, S., Watanabe, M.M., Otsuka, S., Mahakahant, A., Yongmanitchai, W., Nopartnaraporn, N., Liu, Y.D. & Day, J.G.(2002) Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. *International Journal of Systematic and Evolutionary Microbiology* 52: 1577–1595.
http://dx.doi.org/10.1099/ijs.0.01834-0

Szafer, W. (1910) Zur Kenntnis der Schwefelflora in der Umgebung von Lemberg. [Série B] *Bulletin International de l'Académie des Sciences de Cracovie, Classe des Sciences Mathématiques et Naturelles* 1910: 161–167.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
http://dx.doi.org/10.1093/molbev/mst197

Taton, A., Grubisic, S., Brambilla, E., Rutger, D.V. & Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurado Dry Valleys, Antarctica): a morphological and molecular approach. *Applied and Environment Microbiology* 69: 5157–5169.
http://dx.doi.org/10.1128/AEM.69.9.5157-5169.2003

Tillett, D. & Neilan, B.A. (2000) Xanthogenate Nucleic Acid Isolation from Cultured and Environmental Cyanobacteria. *Journal of Phycology* 36: 251–258.
http://dx.doi.org/10.1046/j.1529-8817.2000.99079.x

Villena, M. & Romo, S. (2003) Phytoplankton changes in a shallow Mediterranean lake (Albufera of Valencia, Spain) after sewage diversion. *Hydrobiologia* 506–509: 281–287.
http://dx.doi.org/10.1023/B:HYDR.0000008565.23626.aa

Welsh, H. (1964) A contribution to our knowledge of the blue-green algae of South West Africa. *Nova Hedwigia* 7(1–2): 15–32.

Whitton, B.A. (2011) Cyanobacteria (Cyanophyta). *In*: John, D.M., Whitton, B.A. & Brook, A.J. (Eds.) *The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae*. Cambridge University Press, Cambridge, pp. 31–158.

Zhu, M.L., Yu, G.L., Li, X.C., Tan, W.H. & Li, R.H. (2012) Taxonomic and phylogenetic evaluation of *Limnothrix* strains (Oscillatoriales, Cyanobacteria) by adding *Limnothrix planktonica* strains isolated from central China. *Hydrobiologia* 698(1): 367–374.
http://dx.doi.org/10.1007/s10750-012-1127-8