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REVIEW ARTICLE

Delimitation of the family Oscillatoriaceae (Cyanobacteria) according to the modern polyphasic approach (introductory review)

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Abstract The criteria for cyanobacterial taxonomy have changed substantially in the last several years. The most important of the newly introduced methods are molecular sequencing and the genetic approach, which is considered as a major criterion for the classification of organisms. However, molecular results have not always been in agreement with those of the traditional classification system based on morphological or ecological criteria. Thus, application of the polyphasic approach provides the best means of conducting taxonomic practice, and the coincidences and correlations of various criteria must be discovered. Here are discussed the main problems associated with the taxonomy of simple, relatively wide filamentous cyanobacteria with short vegetative cells, but without prominent cells (heterocytes, akinetes). Examples were selected from the family Oscillatoriaceae, which was traditionally based only on morphological criteria. The main markers for delimitation of this family after revisions and the relations and consequences of various genera are discussed, as well as a proposal to base the final revision of this family according to modern criteria. The members of this cyanobacterial group are distributed in numerous ecosystems around the world and form also distinct communities in tropical America.

Keywords Cytomorphological markers · Molecular methods · Oscillatoriaceae · Polyphasic approach · Taxonomic revision

1 Introduction

Taxonomic classification is the only method that allows recognizing and general reviewing of various groups of organisms around the world. For a long time, the classification of prokaryotic, phototrophic cyanobacteria was based on morphological and (in a few cases) ecological features (cf. Geitler 1932; Elenkin 1938; Starmach 1966; Wehr et al. 2015; and others). The introduction of modern methods, particularly genetic procedures, changed substantially their taxonomic criteria. It is surely useful and necessary to accept different molecular methods (cf. Dvořák 2017), but up to now, only 16S rRNA sequencing has been commonly used and is the most respected method (Ballot et al. 2004; Sciuto et al. 2012; and others). The recognized problem with this approach has been that results from molecular evaluations are not always in agreement with the traditional criteria. The elaboration of two or more parallel classification systems for one taxonomic group is useless and extremely undesirable. The "polyphasic approach" was therefore proposed and introduced, but the methodological prescriptions for this procedure were not defined and they have been interpreted very arbitrarily up to now. This approach should be the combination and synthesis of all important and coinciding criteria and should enable the identification of natural, as well as cultured populations. From this conclusion, it sometimes follows that several morphologically distinct characters, traditionally used for the separation of taxa, are in fact very polymorphic and thus unreliable, while the less distinct cytomorphological markers can be in agreement with the phylogenetic trends. This means that the correlations and coincidences of various and selected cytomorphological features with molecular clades are important and necessary for a correct final classification. Because we

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evaluate living, continually adaptable and variable prokaryotic organisms, the populations, while clearly separated (with hiati in certain characteristics) in one criterion (genetic, cytomorphological, ecological), should also be recognizable in the final system, yielding a scheme of natural diversity.

2 Methods

In the following review, the cyanobacterial family Oscillatoriaceae, representing one clade in the molecular scheme, is evaluated (as an example) by the main components of the polyphasic criteria (Rippka et al. 1981; Anagnostidis and Komárek 1988). The main morphological characters recognized were relatively thick, ± 5–60 µm wide, unbranched, bipolar trichomes without heterocytes and akinetes, and cells shorter than wide. This family was redefined and corrected according to a combination of available modern, mainly genetic features, but also respecting the traditionally known, accepted taxonomic, nomenclatural and terminological principles and rules (principles of priority, typification), prescribed, respected and accepted for all biological systems.

The described generic and subgeneric taxonomic units, classified traditionally in oscillatoriacean cyanobacteria, were included in our study. Particular strains and species were evaluated, of which the characteristics from more methods were available (morphological descriptions, variability of natural populations, isolated strains and their physiological characters and genetic position), and congruencies in these main features were compared. The units (taxa) coinciding at least in two markers and characteristics were evaluated and defined.

The scheme of Oscillatoriaceae sensu stricto (revised) was constructed from our analyses, but the final scheme of this whole family will be published in detail in another place. The nomenclatural designation was accepted with the exact keeping of the taxonomic rules and prescriptions (typification method, principle of priority, etc.). The special evaluation was made by the help of revised strains, which were designated originally by the names of typical species. The strains, identified as corresponding to type species, were particularly studied.

3 Results

Cytology (ultrastructure) – The intracellular structure of the vegetative cells was found to have a good coincidence with different clades, derived from the molecular evaluation of all cyanobacteria. While, e.g., a distinct separation of "unicellular + colonial" versus filamentous cyanobacteria was found independent of the main phylogenetic trends, the main types of organization of thylakoids in cyanobacterial prokaryotic cells have very similar schemes of one type in one and the same clade. Therefore, the basic lines of the modern cyanobacterial system (Komárek et al. 2014) contain both coccoid and filamentous types, but the organization of the thylakoids in the cells is more or less characteristic for one and the same phylogenetic line.

As concerns the oscillatorian genera, all types belonging to this line had either more or less coiled thylakoids in an irregular position or an agglomeration of more or less radially situated thylakoids with certain irregularities (Fig. 1) (Lang and Whitton 1973; Fjerdingstad et al. 1976). Rarely are the thylakoids more aggregated in the cell periphery. It is possible that the differences in detail will be in agreement with the lower, subfamiliar taxonomic units, but we do not yet have materials or data for this evaluation. However, the common scheme of thylakoid arrangement is possible to designate as characteristic for the whole family. The filamentous species with thylakoid patterns distinctly different from this oscillatoriacean type (and usually with thinner trichomes) must be evidently eliminated from this clade and from the family Oscillatoriaceae.

Various other intracellular inclusions, and also a few modifications in the cell walls (calyptras, necridic cells), occur in oscillatoriacean cells. However, they were still published only few data about intracellular structures, which enable to define the taxonomic value of this family.

Morphology – Various, morphologically different and distinguishable taxa were traditionally included to the separated oscillatoriacean units (genera, species). The main and commonly used determining features of the whole group were the formation of uniseriate, bipolar trichomes and the absence of prominent cells (heterocytes, akinetes). However, the main diacritical features of the family Oscillatoriaceae, corresponding with the modern concept are illustrated in Fig. 2. One from important criteria between genera inside the family was traditionally used only the presence of sheaths (without sheath = *Oscillatoria* Vaucher ex Gomont, with formation of a facultative sheath under distinct conditions = *Phormidium* Kützing ex Gomont, occurrence of an obligatory firm, often lamellated sheath = *Lyngbya* C. Agardh ex Gomont; comp. Figure 3). However, this character cannot be used without exceptions and it is usable with certain reservation in few cases only. Few other small generic units were separated from the three main genera by special other distinct cytomorphological characters (with gas vesicles in cells—*Aerosakkonema* Thu et M.M. Watanabe, *Limnoraphis* Komárek et al., with false branching in ensheated types—*Plectonema* Thuret ex Gomont). Other factors, which were used

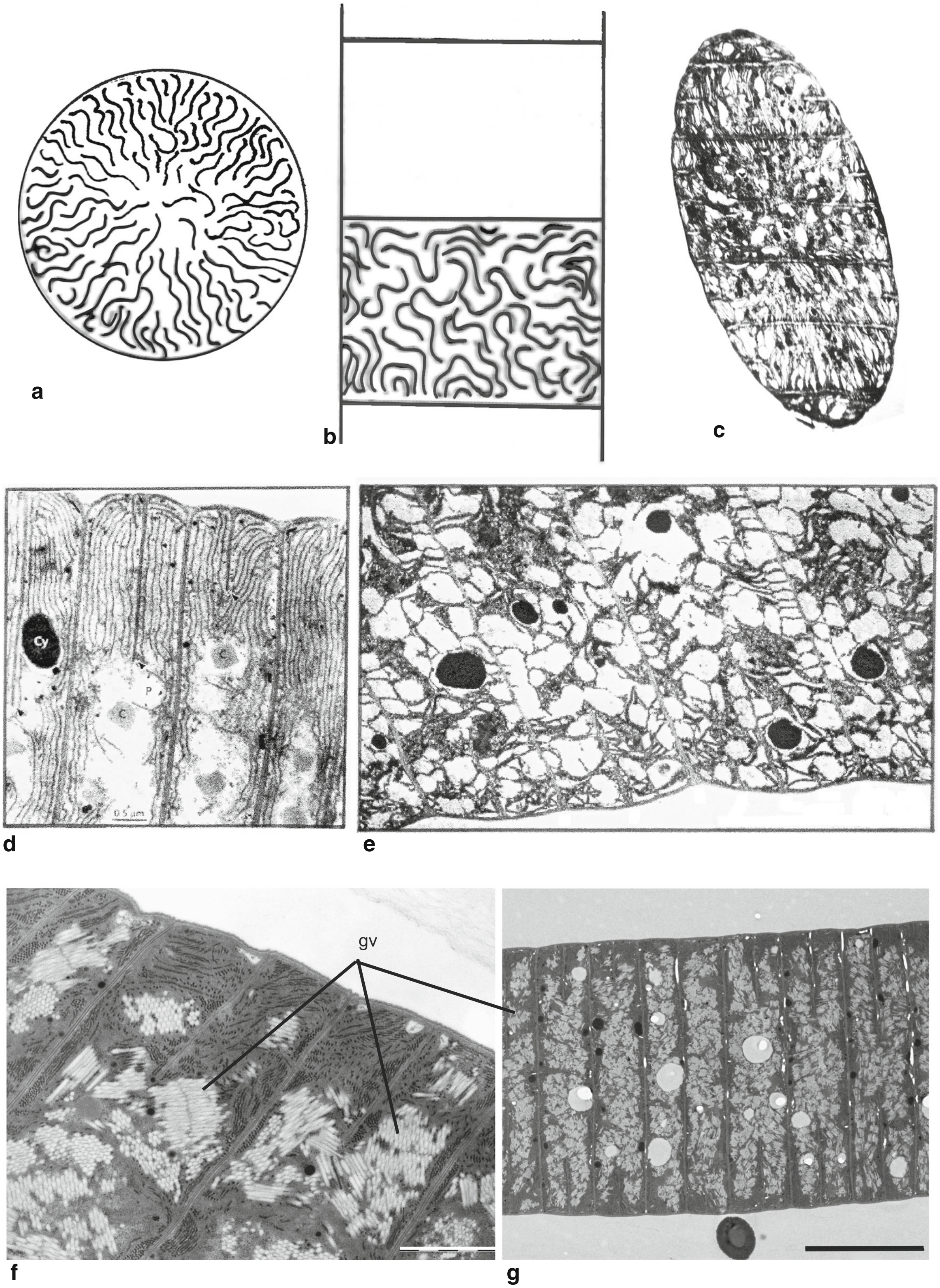


Fig. 1 Position of thylakoids in oscillatoriacean genera: **a**, **b** = scheme of a cross section (**a**) and lengthwise section (**b**) of an oscillatoriacean trichome (after Anagnostidis and Komárek 1988); **c** = tangential section of the trichome of *Oscillatoria limosa* (after Fjerdingstad et al. 1976); **d** = part of a lengthwise section of the trichome of *Oscillatoria* sp. (after Lang and Whitton 1973); **e** = part of a lengthwise section of the trichome of *Blennothrix ganeshii* (after Watanabe and Komárek 1989); **f**, **g** = lengthwise sections of trichomes of *Limnoraphis robusta* (orig. Šmarda), with fascicles of thylakoids and gas vesicles (gv); scale bars **f** = 1 µm, **g** = 5 µm

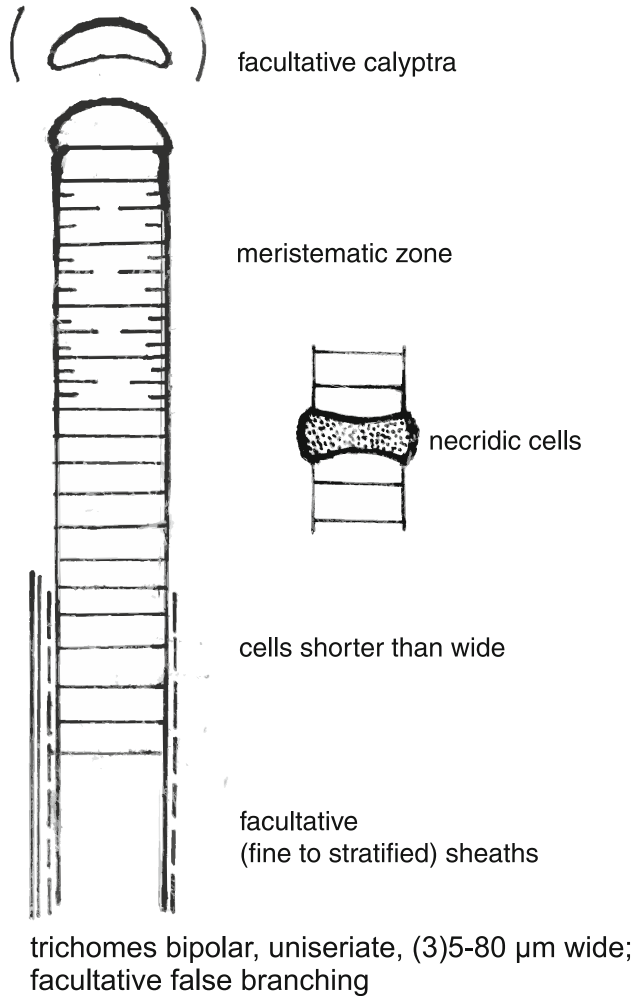


Fig. 2 Main morphological markers of Oscillatoriaceae

to taxonomic delimitation of genera, and from which few were sometimes neglected, were, e.g., the trichome width, the ultrastructure of cells, the ratio of cell length to width, or several important biological markers, e.g., the type of fragmentation of the trichomes and hormogonia formation, existence of meristematic zones and type of necridic cells (Watanabe and Komárek 1989; Ballot et al. 2004; Malone et al. 2015).

After application of genetic (molecular) criteria, the taxonomic value of several characters had to be reevaluated with the result that several special, different types must be removed and separated from the oscillatoriacean family (Komárek et al. 2014). It is interesting that the corrected concept of Oscillatoriaceae contains the main generic markers of all three basic genera with respect to their type species (*Oscillatoria princeps* Vaucher ex Gomont, *Phormidium lucidum* Kützing ex Gomont, *Lyngbya confervoides* C. Agardh ex Gomont) (Fig. 3, Table 1). (The type of *Phormidium* was not yet studied by molecular analyses and the typification particularly of this genus and other separated taxonomic groups must be confirmed; the respective results up to date are available in the Fig. 3.) This whole oscillatoriacean group, represented by the three basic mentioned generic types, is therefore clearly and distinctly characterized also morphologically (morphology and structure of trichomes). The results are included in the summary and final description of the family, and numerous species, which do not correspond to the revised combined genetic-morphological concept, must be transferred to other lines and taxa.

Life cycles – The reproduction and life cycles of the revised oscillatoriacean cyanobacteria are simple. The cells divide regularly in filaments crosswise during the vegetation season, and trichomes disintegrate usually by help of necridic cells (Geitler 1932; Elenkin 1938; Starmach 1966; Fjerdingstad et al. 1976). However, they are not yet known well the factors, influencing this process. Meristematic zones were discovered in several types, which occur often only on a part of a trichome (Fig. 2). In these areas, the crosswise division of cells in certain segments of the trichomes proceeds in a rapid sequence and sometimes there are cases when the following crosswise separation will start to develop still before the closing of the previous cross wall. The details of this whole process and the events, influencing the formation of meristematic zones are unclear. However, present knowledge indicates that the formation of meristematic zones is characteristic only for a few cyanobacterial groups, including Oscillatoriaceae. The meristematic zones in the other types, eliminated from the traditional wide oscillatoriacean family, were either not described with certainty, or they are of another character.

The trichomes in Oscillatoriaceae divide intensely in numerous hormogonia (which are sometimes relatively short, containing only several cells), in several cases separated by necridic cells. However, the factors (ecophysiological and biochemical parameters) influencing this process are also not yet well known.

Ecology – The oscillatoriacean cyanobacteria occur in wide ecological situations in numerous ecosystems. However, the revised oscillatoriacean group has certain ecological limits. The different types and species were found mostly in benthic and periphytic habitats, in both marine and freshwater ecosystems. Interesting are the few planktic types (usually with gas vesicles in cells), or special types from extremely mineral or extreme temperature dependent localities. However, the whole group is possible to designate as aquatic-periphytic, from which the only a few types are adapted to other related habitats with special, unusual conditions (thermal waters, plankton, etc.).

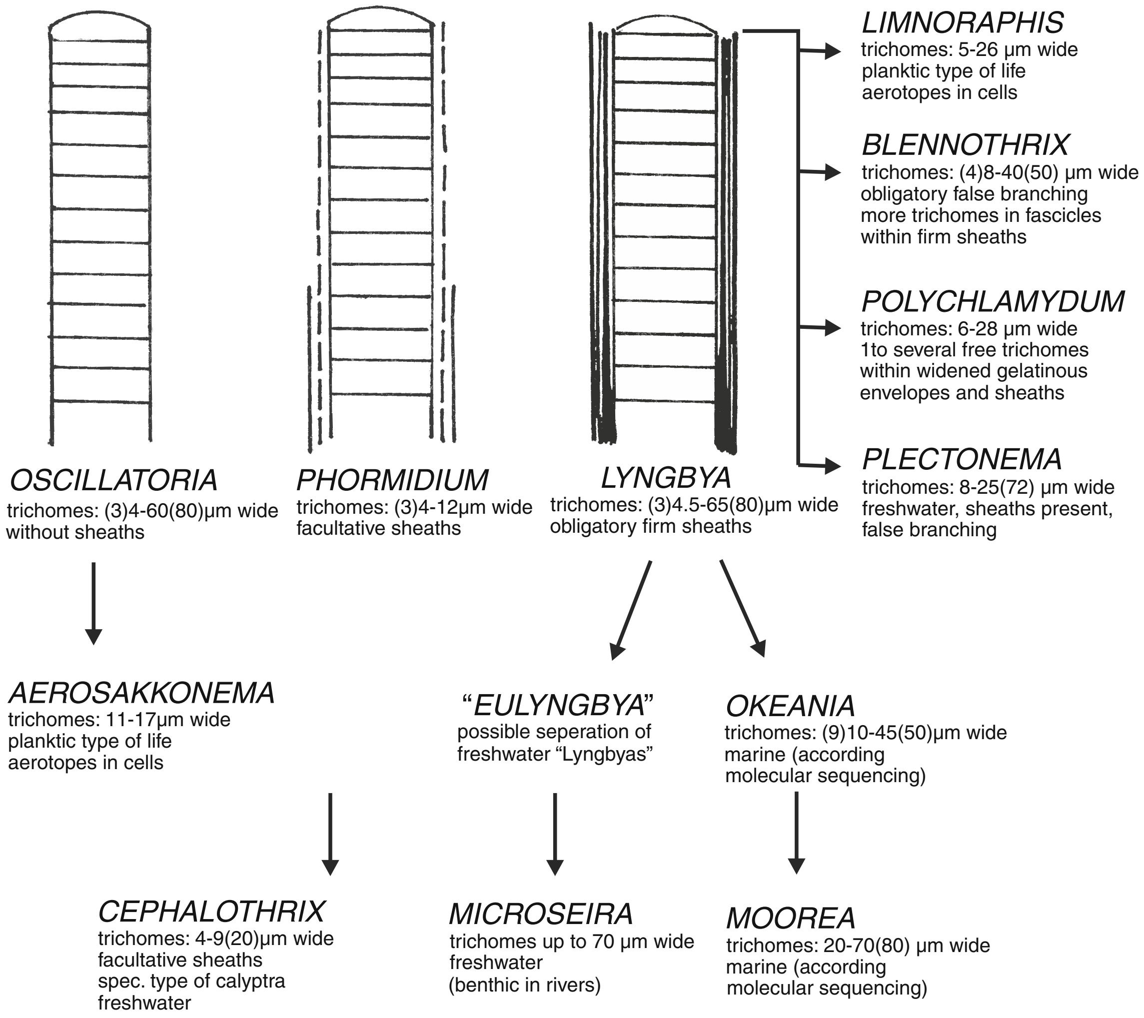


Fig. 3 Cytomorphological characters of the present revised genera of Oscillatoriaceae after polyphasic evaluation

Molecular sequencing – Molecular evaluation must be used as the primary criterion for classification of organisms and the revised taxonomic units should belong to one genetic clade. 16S rRNA sequencing and ITS are up to now mostly used for cyanobacteria, and these methods are considered as decisive for further taxonomic treatment (Rippka et al. 1981; Sciuto et al. 2012; Komárek et al. 2014). Of course, more detailed or complete genetic characterizations of various species are desirable, but the corrected taxonomic units should be always well characterizable with this primary evaluation. The other markers (cytomorphology), which are congruent with the molecular basis, have to be redefined and corrected (cf. Komárek and Kaštovský 2003). Moreover, we have the molecular data of all known intrageneric taxa of single genera only very rarely.

The greatest methodological problem is therefore that only selected isolated strains from the wide genera are commonly used for molecular sequencing (Ballot et al. 2004; Sciuto et al. 2012; Malone et al. 2015; and others). Very questionable are also the designations used for comparable strains in phylogenetic trees, because the authors do not correct nor revise and reevaluate the introductory material. The resulting relations and conclusions from such constructed phylogenetic trees have therefore many anomalies, if only the original designations are used without taxonomic control and such conclusions are evidently wrong. Large taxonomic revisions of all the strains, used in the construction of phylogenetic trees, are therefore very desirable (but time-consuming, of course). In ecological, hydrobiological and floristic studies, the authors need simple identification manuals, enabling identification of all possible taxa in the studied localities. According to our experience, the morphological markers, used for the determination of strains in natural material are well coincident with the phylogenetic lines and are acceptable, but only if the morphology of all included strains is carefully revised and corrected, and the coinciding characters are defined. The genera and species must be therefore unique both phylogenetically and morphologically.

4 Conclusions and perspectives

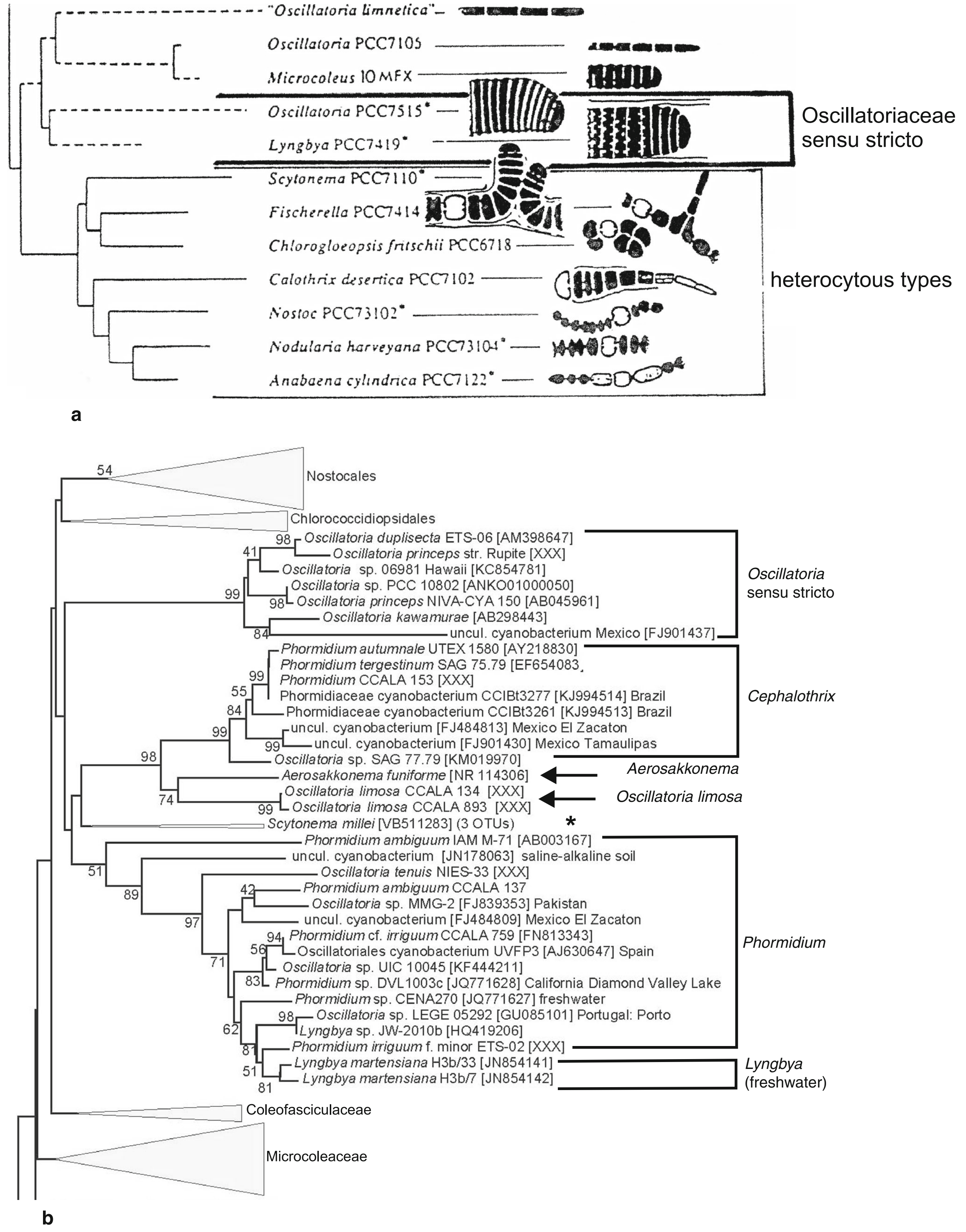
From the previous data follows the wide set of molecular and cytomorphological features, which depend on one another. Their synthesis represents the list of characters typical for the cyanobacterial groups, including the revised family Oscillatoriaceae (polyphasic evaluation). The molecular (genetic) criteria are used as a bases for the definition of this family, and the revised morphological description and taxonomic concept of Oscillatoriaceae contains the following markers (comp., e.g., Moro et al. 2007; Komárek et al. 2014; and others; Fig. 3):

- Simple, relatively wide (3–5–60–80 μm), uniseriate and bipolar trichomes.
- Cells always distinctly shorter than wide.
- Irregular or radially irregular arrangement of thylakoids (Fig. 1).
- Crosswise division of cells, sometimes in a rapid sequence in some parts of the trichomes.
- Ability to form meristematic zones in the trichomes (Fig. 2).
- Facultative occurrence of mucilaginous envelopes, sheaths or laminated sheaths.
- Possibility of false branching in ensheathed genera, sometimes with the occurrence of more trichomes in one sheath.
- Uniformity and relations within one clade in molecular phylogenetic trees.
- Absence of prominent cells (heterocytes, akinetes).
- Aquatic and subaquatic, mostly periphytic (rarely planktic) environments.

Fig. 4 Examples of molecular relations and position of several oscillatoriacean genera in phylogenetic trees, constructed according to data from GenBank and corresponding to the revised family Oscillatoriaceae: **a** separation of the family Oscillatoriaceae sensu stricto from other cyanobacterial lines (after Komárek and Kaštovský 2003); **b** relations of several oscillatoriacean genera according to the 16S rRNA sequencing (derived from GenBank, orig. Strunecký), * = in the strain VB511283, designated as "*Scytonema millei*" never were found heterocytes (this strain belongs evidently to the typical cluster of the genus *Plectonema*) ▶

Table 1 Genera of the revised family Oscillatoriaceae after polyphasic evaluation

Genus	Type species	Approx. number of morphospecies	Presence of sheaths	Gas vesicles (aerotopes)	Special differential characters (comp. Fig. 3)
Aerosakkonema	*A. funiforme* Thu et Watanabe in Thu et al.	1	–	+	Planktic, solitary trichomes
Blennothrix	*B. vermicularis* Kützing ex Anagnostdis et Komárek	20–25	+	–	More trichomes in one sheath
Cephalothrix	*C. komarekiana* Malone et al.	2	(+)	–	Facultative sheaths, calyptrate trichomes
Limnoraphis	*L. hieronymusii* (Lemmermann) Komárek et al.	4	+	+	Planktic, solitary trichomes
Lyngbya (marine)	*L. confervoides* Agardh ex Gomont	30–40	+	–	Obligatory firm sheaths, marine
Lyngbya (freshwater)	*L. martensiana* Meneghini ex Gomont	80–90?	+	–	Obligatory firm sheaths, not marine
Microseira	*M. wollei* (Farlow ex Gomont) McGregor et Sendall	1	+	–	False branching
Moorea	*M. producens* Engene et al.	2	+	–	Marine, sheaths, isolated molecular line
Okeania	*O. hirsuta* Engene et al.	5	+	–	Marine, sheaths, isolated molecular line
Oscillatoria	*O. princeps* Vaucher ex Gomont	100–110?	–	–	Total absence of sheaths
Phormidium	*P. lucidum* Kützing ex Gomont	8–10	(+)	–	Facultative sheaths
Plectonema	*P. tomasinianum* Bornet ex Gomont	8–10	+	–	Sheaths, obligatory false branching
Polychlamydum	*P. insigne* West et West	9?	Gelat. envel.	–	Gelatinous envelopes around trichomes


"Oscillatoria limnetica"
Oscillatoria PCC7105
Microcoleus 10 MFX
Oscillatoria PCC7515*
Lyngbya PCC7419*
Scytonema PCC7110*
Fischerella PCC7414
Chlorogloeopsis fritschii PCC6718
Calothrix desertica PCC7102
Nostoc PCC73102*
Nodularia harveyana PCC73104*
Anabaena cylindrica PCC7122*
Oscillatoriaceae sensu stricto
heterocytous types
a
54
Nostocales
Chlorococcidiopsidales
98
Oscillatoria duplisecta ETS-06 [AM398647]
41
Oscillatoria princeps str. Rupite [XXX]
Oscillatoria sp. 06981 Hawaii [KC854781]
99
Oscillatoria sp. PCC 10802 [ANKO01000050]
98
Oscillatoria princeps NIVA-CYA 150 [AB045961]
Oscillatoria kawamurae [AB298443]
84
uncul. cyanobacterium Mexico [FJ901437]
Oscillatoria sensu stricto
Phormidium autumnale UTEX 1580 [AY218830]
Phormidium tergestinum SAG 75.79 [EF654083]
99
Phormidium CCALA 153 [XXX]
55
Phormidiaceae cyanobacterium CCIBt3277 [KJ994514] Brazil
84
Phormidiaceae cyanobacterium CCIBt3261 [KJ994513] Brazil
99
uncul. cyanobacterium [FJ484813] Mexico El Zacaton
99
uncul. cyanobacterium [FJ901430] Mexico Tamaulipas
98
Oscillatoria sp. SAG 77.79 [KM019970]
Cephalothrix
Aerosakkonema funiforme [NR 114306]
Aerosakkonema
74
Oscillatoria limosa CCALA 134 [XXX]
99
Oscillatoria limosa CCALA 893 [XXX]
Oscillatoria limosa
Scytonema millei [VB511283] (3 OTUs)
*
Phormidium ambiguum IAM M-71 [AB003167]
uncul. cyanobacterium [JN178063] saline-alkaline soil
51
Oscillatoria tenuis NIES-33 [XXX]
89
42
Phormidium ambiguum CCALA 137
Oscillatoria sp. MMG-2 [FJ839353] Pakistan
97
uncul. cyanobacterium [FJ484809] Mexico El Zacaton
94
Phormidium cf. irriguum CCALA 759 [FN813343]
56
Oscillatoriales cyanobacterium UVFP3 [AJ630647] Spain
71
Oscillatoria sp. UIC 10045 [KF444211]
83
Phormidium sp. DVL1003c [JQ771628] California Diamond Valley Lake
Phormidium sp. CENA270 [JQ771627] freshwater
Phormidium
98
Oscillatoria sp. LEGE 05292 [GU085101] Portugal: Porto
62
Lyngbya sp. JW-2010b [HQ419206]
81
Phormidium irriguum f. minor ETS-02 [XXX]
51
Lyngbya martensiana H3b/33 [JN854141]
81
Lyngbya martensiana H3b/7 [JN854142]
Lyngbya (freshwater)
Coleofasciculaceae
Microcoleaceae
b

- Reproduction by hormogonia, developing after formation of morphologically special necridic cells.
- The genera which belong to the family Oscillatoriaceae according to the present revised concept are included in Fig. 3 and Table 1.

This cluster, which is defined also morphologically, results from the phylogenetic trees. However, if we accept the enormous number of other, arbitrarily selected data from GenBank and designate by traditional names the oscillatoriacean taxa, we can obtain various trees with the position of *Oscillatoria*, *Phormidium* and *Lyngbya* strains in very different places. However, the phylogenetic trees are not diverse after elimination of types, which do not evidently belong to the Oscillatoriaceae sensu stricto after our revision (comp. Fig. 4; Ballot et al. 2004; Sciuto et al. 2012; and others). The revised family Oscillatoriaceae can be therefore well defined molecularly, as well as by the definition of its differential cytomorphological characters.

The methodological guidelines for the application of methods for a satisfactory taxonomic treatment of cyanobacteria are not yet clear and must be defined step by step after future studies. Occurrence of different species of the same genus or designations of different strains with the same generic names in different and distant phylogenetic lines are evidently the marker of wrong identification and designation and requires wide methodological and morphological control.

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