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RE-EVALUATION OF THE RELATIONSHIPS WITHIN SOME EGYPTIAN SPECIES OF SOIL CYANOBACTERIA

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ABSTRACT

In this study, morphological and ecological characteristics of three Egyptian Cyanophyceae genera; *Oscillatoria* (five species), *Lyngbya* (three species) and *Anabaena* (three species) were analyzed using numerical taxonomic system of multivariate statistical program. Data were analyzed by clustering method and similarity coefficients using NTSYSpc version 2.02i. Three different phenograms were produced for the studied genera and the relationships between the species were discussed.

Keywords: soil cyanophyceae, *Oscillatoria*, *Lyngbya*, *Anabaena*, numerical analysis, cluster method, species relationships, Egypt.

INTRODUCTION

Attempts to apply the numerical classification of Blue-green algae = Cyanobacteria (Kurt *et al*., 2010) using numerical taxonomic system of multivariate statistical program are almost hardly rare in Egypt. In soil algae, the system of classification has based largely on morphological characters and on some ecological data which have evolved over many years ago. In some cases, established genetic basis data as well as the few morphological characters available are insufficient to distinguish between organisms or speak about their relationships (Robert, 1973). Attempt to apply the numerical taxonomy system program attracted us as the calculated simple matching coefficients and a drawn phenogram might solve the imbalance of many keys and reach to true relationships between species of same genus. Sometimes, these relationships cannot be touched through the taxonomic algal keys.

Recently, Hoffmann *et al*. (2005) proposed a revision to the cyanobacterial classification under the botanical code on the basis of evolutionary (genetic) and ecological relations, as well as phenotypic variation. Wacklin (2006) confirmed that the current classification used for cyanobacteria is based mainly on morphology. In many cases, the classification is known to be misfit with the phylogeny of cyanobacteria. The evaluation of this classification is complicated by the fact that numerous strains are only described morphologically and have not been isolated. Moreover, the phenotype of many cyanobacterial strains alters during prolonged laboratory cultivation. Wacklin *et al*. (2009) explained that there are some species which are consistent with the phenotypic variation but the others do not consistent. The modern approach of cyanobacterial classification must apply all available and important methodological procedures and criteria in taxonomic analyses and the classification must respect all knowledge about the organisms and their evolutionary relations (Komárek, 2005).

The relationships between different species are not yet definitely solved, but the separation of algal genera is justified and all up to date definitely described (Wacklin *et al*., 2009).

MATERIALS AND METHODS

Culturing and isolation of algae

The following methods were applied on air-dried soil samples collected from different sites in Kafr El-Sheikh governorate (El-Gamal *et al*., 2008), using Z-medium (Staub, 1961) and modified Watanabe medium (El-Nawawy *et al*., 1958) for isolation and culturing of cyanobacteria. Semi-solid medium as described by El-Ayouty and Ayyad (1972) and filter paper method as recommended by Esmarch (1914) were applied. About 10 g of the soil sample were also placed in flasks containing 100 ml of the sterilized liquid medium. The Petri-dishes as well as flasks were incubated at 30°C under continuous light (2500 lux). The identification of algae was carried out using the following criteria: Thallus color, thallus morphology and dimension, size of heterocyst, vegetative and reproductive cells. Heterocyst-forming blue-green algae were also cultured in nitrogen-free Z-medium (El-Gamal, 1995). The studied species are shown in Figure-1.

Numerical analysis

The presence or absence of each of the different character was treated as a binary character in a data matrix (coded 1 and 0, respectively) for computation. Data were analyzed by using clustering method and similarity coefficients between the studied species using NTSYSpc version 2.02i (Rohlf, 1998).

RESULTS AND DISCUSSIONS

Taxonomy is the science of classification and has several functions. The first is to describe the species which is the basic taxonomic unit. The second is to catalogue these species into some arrangement enabling the relationships between species to be recognized. The taxonomy of Cyanobacteria was substantially changed in last decades of 20th century. Modern ecological investigations, electron microscopy and particularly

introduction of molecular methods influenced our knowledge and consequently, classification of cyanobacterial diversity (Komárek, 2005).

Our data aim to quantify the relationships between living organisms on the basis of the numbers of criteria they share. Cluster analysis statistics method was used to determine the taxonomic value of certain characters and the affinities between the species. Hence, the obtained phenogram expresses the same and different traits between species. In this type of classification, individual characters gain a significant value not given to them in hierarchical taxonomy. The great advantage of this program (the NTsys program) is that the rapid categorization of species through the presence of many characteristics for comparison. For the numerical analysis, the algal species were numbered. Each species had a unique code number to facilitate the running of computation program as summarized in Table-1.

Numerical analysis of morphological and ecological characters

The morphological and ecological criteria of the examined species (Figure-1) have been considered as attributes and are used for a number of numerical analyses using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Twenty, twelve and twenty six characters have been recorded for *Oscillatoria*, *Lyngbya* and *Anabaena*, respectively. The data of the morphological and ecological characters were used in the numerical analysis were shown in Tables 2, 3 and 4 (respectively).

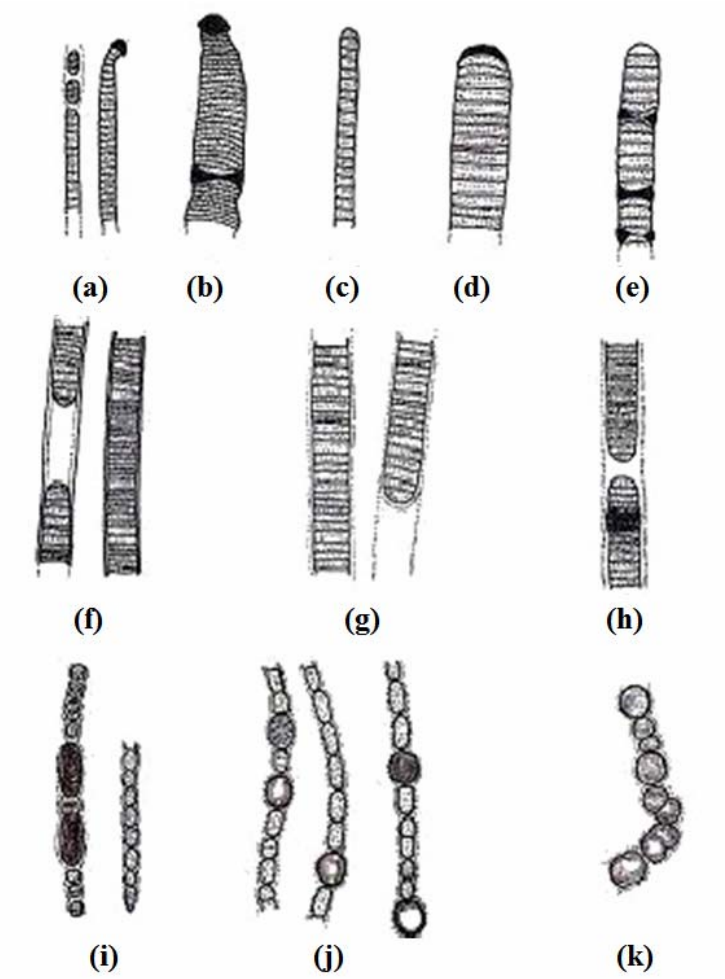


Figure 1: (a) *Oscillatoria amoena* (*X* 880), (b) *O. anguina* (*X* 1120), (c) *O. nigra* (*X* 880), (d) *O. sancta* (*X* 880), (e) *O. subbrevis* (*X* 1100), (f) *Lyngbya birgei* (*X* 400), (g) *L. hieronymusii* (*X* 880), (h) *L. martensiana* (*X* 880), (i) *Anabaena torulosa* (*X* 1120), (j) *A. wisconsinense* (*X* 1500) and (k) *A. variabilis* (*X* 2200).

Table-1. Summarizes the algal species and their code numbers in the present study.

Species code number	Algal species
1	*Oscillatoria amoena*
2	*O. anguna*
3	*O. nigra*
4	*O. sancta*
5	*O. subbrevis*
6	*Lyngbya birgei*
7	*L. hieronymusii*
8	*L. martensiana*
9	*Anabaena torulosa*
10	*A. wisconsinense*
11	*A. variabilis*

Table-2. Comparative recordings of 20 morphological and ecological characters of *Oscillatoria* species.

Characters	Attributes	Species code numbers*
Trichome color	Blue-green	1
	Dark-green	2
	Blackish green	3
	Dark gray-green	4
	Pale gray-green	5
Cell diameter	3.6-4.8 µ	1
	4.8-6 (-6.6) µ	5
	(7.2-) 7.8-9.6 µ	3
	8.9-9 µ	2
	(10.8-) 12-13.2 µ	4
Cell length	1.2-1.8 µ	1
	1.2-2.4 µ	5
	3.6-4.8 µ	4
	4.2-4.8 µ	1 and 2
Apical cell	Capitate	1 and 2
	Calyptrate	1, 2 and 4
Constriction at the cross wall	Constricted	1 and 2
Granulation of cell	Granulated	1, 2 and 4
Trichome aggregation	Solitary	5
Organic matter level	High	3 and 4

* Numbers and names of species are listed in Table-1.

Table-3. Comparative recordings of 12 morphological and ecological characters of *Lyngbya* species.

Characters	Attributes	Species code numbers*
Filament color	Dark blue-green	6 & 8
	Light blue-green	7
Cell contents	Granulated	7 & 8
Cell shape	Short shaped	6 & 8
	Cylindrical shaped	7
Cell diameter	8.4- 9.6 µ	8
	10.8-11.4 µ	7
	19.2- 21.6 µ	6
Cell length	(1.8-) 2.4- 3 µ	8
	3.6- 4.8 µ	7
	3.6- 5.4 (-6) µ	6
Salinity	Present	7

* Numbers and names of species are listed in Table-1.

Table-4. Comparative recordings of 26 morphological and ecological characters of *Anabaena* species.

Characters	Attributes	Species code numbers*
Trichome color	Light blue-green	9
	Gray-green	10
	Dark blue-green	11
Cell shape	Barrel-shaped	9
	Quadrate to cylindrical	10
	Compressed globose	11
Cell diameter	(3-)3.6-(-4.2) µ	10
	3.6-4.8 µ	11
	(3.6-)-4.8-6 µ	9
Cell length	4.4-8.4 µ	9
	4.8- 6 (-8.4) µ	10
	8.4-10.8 µ	11
Heterocyst shape	Round avoid	9
	Depressed globose	10
	Globular	11
Heterocyst diameter	(3.6-) 4.2-4.8 µ	10
	(4.8-) 6-7.8 µ	9
	4.8-6 µ	11
Spore diameter	(6.3-) 7.2-8.4 µ	10
	7.2- 8.4 (-9.6) µ	11

	8.4-10.8 µ	9
Spore shape	Oblong	9
	Oval to globular	10
	Ovate	11
Salinity	Present	9 & 11
Organic carbon	present	9 & 11

* Numbers and names of species are listed in Table-1.

The phenogram produced (Figure-2) by the analysis of the studied species of the genus *Oscillatoria* based on coding of 20 attributes shows that the examined species have a total similarity coefficient of about 1.48. At this level, *O. subbrevis* (5) was split off from the other species and then at 1.42 levels, the remaining species are separated into two groups. The first one composed of *O. nigra* (3) and *O. sancta* (4), which they distinguished from each other at the 1.32 level. The other group included *O. amoena* (1) and *O. anguina* (2), which they distinguished from each other at the 1.19 level.

According to Prescott (1982), the five species of *Oscillatoria* were separated based on the size of trichome whereas; *O. scanta* was greater than 10 µ, while the other species was less than 10 µ. It was seemed that both *O. amoena* and *O. subbrevis* are closely related to each other. While, *O. anguina* and *O. nigra* were seemed as if they were close together. The Prescott key is adopted in part, on the color of trichome to differentiate between species.

In classical taxonomy of Cyanobacteria, cell size plays an important role at the species level, but under specific environmental conditions, the cell size of most species is influenced. Kondratyeva *et al*. (1974) measured cell size of *Microcystis aeruginosa* and found that larger cells occurred under less favorable culture conditions. They pointed out that underestimation of the effect of environment on cell size may cause errors in recognition of intraspecific taxa. Thus, the use of possible taxonomic criteria in finding new relationships between living organisms is necessary to correct a lot of relationships.

The phenogram produced by the analysis of the studied species of *Lyngbya* based on a total of 12 characters is shown in Figure-3. The phenogram obtained shows that the examined species of *Lyngbya* have a total similarity coefficient of about 1.54. At this level, *L. hieronymusii* (7) was split off from the other two species and then at the level of 1.11, *L. birgei* and *L. martensiana* (6 and 8) were distinguished from each other.

Prescott (1982) has based on the filament diameter in the separation of the three species of *Lyngbya* and accordingly, he has separated *L. birgei* from *L. hieronymusii*. In addition, Prescott depended on the environmental conditions associated with *Lyngbya* species to separate *L. martensiana*. Accordingly, there was no clear close relationship between the three algal species.

On the other hand, the phenogram produced by the analysis of the studied species of *Anabaena* based on a total of 26 characters is shown in Figure-4. The resulting phenogram shows that the examined species of *Anabaena* have a total similarity coefficient of about 1.45. At this level, *A. wisconsinense* (10) was split off from the other two species and then at the level of 1.33, *A. torulosa* and *A. variabilis* (9 and 11) were distinguished from each other.

Following Prescott (1982), the classification of the three species of *Anabaena* was depended on their nature in the aquatic environment as euplanktons or otherwise, therefore *A. wisconsinense* and *A. torulosa* could be separated from each other. On the other side, *A. torulosa* was separated from *A. variabilis* depending on the presence of akinetes near or far from heterocysts. Therefore, *A. torulosa* and *A. variabilis* had the same path during identification, while *A. wisconsinense* had a different route. Ezhilarasi and Anand (2010) categorized ten *Anabaena* isolates into two major clusters. One of them put *A. torulosa* and *A. variabilis* in the same path according to protein profiles pattern. The present data are in agreements with the findings of Prescott's key study (1982) and Ezhilarasi and Anand (2010).

The use of programmed systems like NTsys program may explain the real relationships between species using many of the characteristics of the species and may reduce the problems that correspond to the taxonomists during the classification of species. It is concluded that both field and culture information are useful in enhancing our knowledge of cyanobacterial systematics. The main problems met in applying morphological criteria in Cyanophyta classification arise from the considerable variability in morphological features within different environmental conditions (Komárek, 1991).

Figure 2: UPGMA-phenogram constructed for the five species of the genus *Oscillatoria* based on coding of 20 characters.

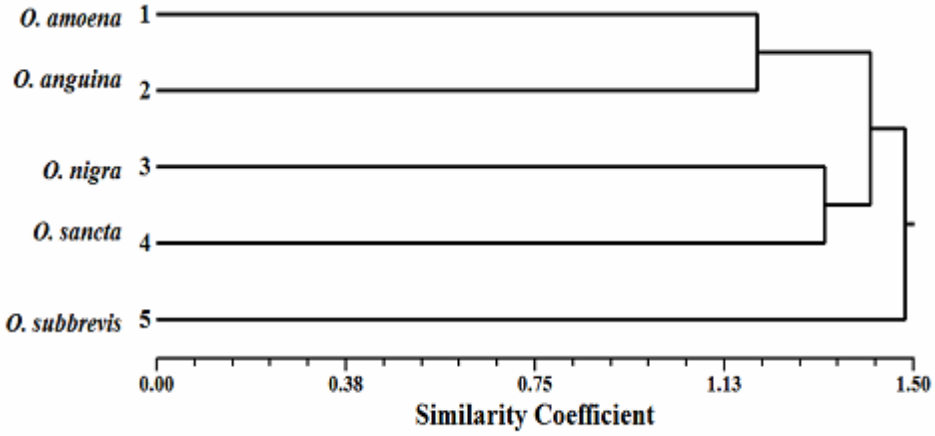


Figure 3: UPGMA-phenogram constructed for the three species of the genus *Lyngbya* based on coding of 12 characters.

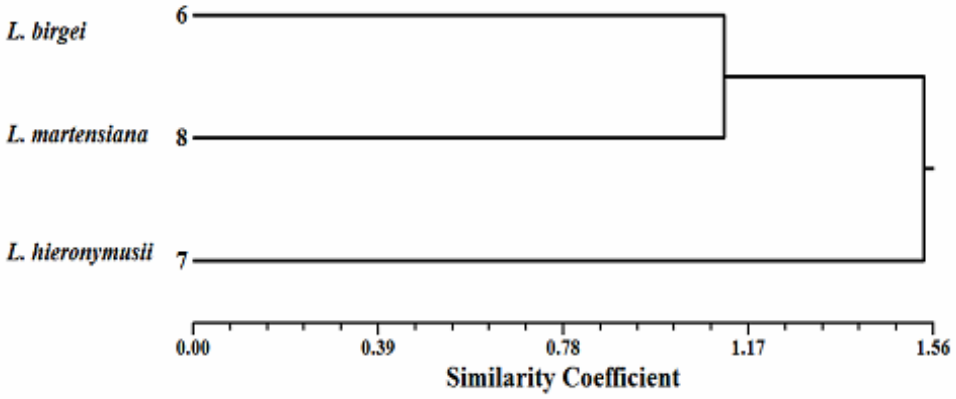
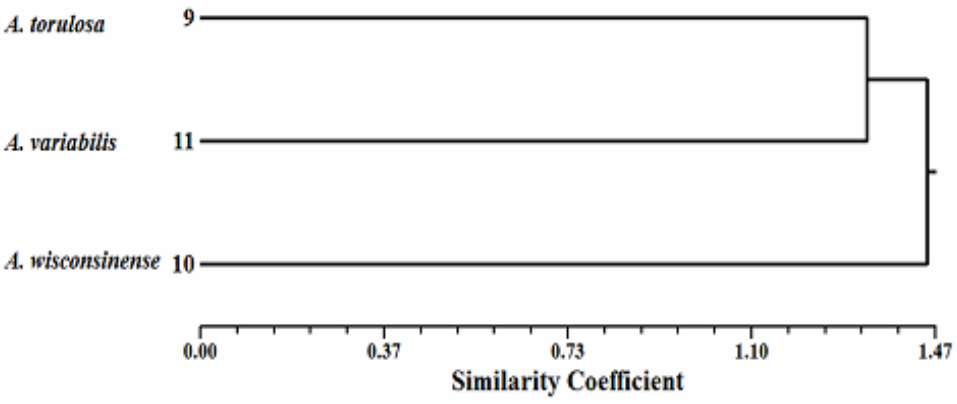


Figure 4: UPGMA-phenogram constructed for the three species of the genus *Anabaena* based on coding of 27 characters.



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