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New Records of Nostocalean Cyanobacteria from Rice Fields in the Golestan Province in North-East of Iran

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The algal flora of five stations from paddy-fields of Golestan Province was investigated between October 2006 and September 2007. Eight filamentous heterocystous Nostocacean are recorded here for the first time from paddy fields of Iran: *Cylindrospermum licheniforme*, *Cylindrospermum stangnale*, *Cylindrospermopsis raciborskii*, *Calothrix ghosei*, *Gloeotrichia longiarticulata*, *Aulosira implexa* var. *crassa*, *Tolypothrix conglutionata* var *colorata*, *Scytonema multiramosum*. *Cylindrospermum stagnale*, *Aulosira implexa*, *Scytonema multiramosum*, *Tolypothrix conglutionata* were dominant species in all stations and seasons, but *Gloeotrichia longiarticulata* and *Cylindrospermopsis raciborskii* were observed mainly during the spring and summer. Morphological characteristics of these species are described in detail and information about their ecological distribution is given. Vol. 1, No. 2, 50-55.

Key Words: Cyanobacteria, Golestan Province, Paddy field, Taxonomy, New records.

Introduction

Cyanobacteria are organisms that have characteristics of both simple plants and bacteria (Ahoren, 2004). They are photosynthetic prokaryotes that carry out an oxygen–evolving photosynthesis virtually identical to that of higher plants.

Cyanobacteria are present abundantly in rice fields and are important in helping to maintain the fertility of these fields by performing nitrogen fixation and producing various nitrogenous and non-nitrogenous compounds (Shokravi *et al*., 2007). The soil of many rice fields contains a high density of cyanobacteria, and over 50% of cyanobacterial genera in the rice paddy fields are heterocystous filamentous bacteria (Jeong–Dong and Choul–Gyun, 2008). The layer of water in the fields and the nutrient conditions of the soil define a suitable condition for growth of blue-green algae (Santra, 1993). Heterocystous Cyanobacteria are traditionally considered as cosmopolitan microorganisms with remarkable capabilities to acclimatize to broad ranges of environmental conditions (Hickman, 1978). They have been tremendously important in shaping the course of ecological change throughout earth's history (Saadatnia and Riahi, 2009).

Heterocystous cyanobacteria classified as Nostocales are characterized by shape, size, and color of filaments, as well as features of trichomes, cells, and spores. Genera of the Nostocales exhibit the highest degree of morphological complexity and differentiation within the cyanobacteria .(Anognostidis and Komárek, 1990).

The Golestan Province in Iran lies between latitudes of 36°15′ and 38°15′ N and longitudes of 54°30′ and 56°30′ E. The paddy fields of Golestan Province encompassed nearly 62000 hectares of cultivation fields last year. Golestan is among the most important rice producing provinces in Iran. The study on Nostocacean Flora of rice fields may considerably enhance yield within the area.

It has previously been reported that some strains of cyanobacteria, especially *Aulosira*, *Scytonema*, *Cylindrospermum,* are common in the paddy fields of northern Iran, specially in Golestan Province (Shokravi *et al*., 2002). However, a detailed morphological and taxonomic study of the cyanobacteria in Iran has not been per-

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formed. Our current knowledge of soil algae is based mainly upon enrichment culture studies, however selective culture conditions may have caused bias in the resulting species lists.
The aim of this paper is to expand the existing knowledge on the algal flora of paddy fields of Golestan Province, with a focus on cyanobacteria.

Material and methods

Soil samples were collected from paddy fields of Golestan province. Five stations were chosen for sampling: Kordkoy (36˚49´ N, 54˚ 07´ E), Gorgan (36˚50´ N, 54˚ 27´ E), Aliabad (36˚53´ N, 54˚ 52´ E), Azadshahr (37˚06´ N, 55˚ 10´ E), and Minodasht (37˚13´ N, 55˚ 22´ E). The samples were taken from non-flooded soils from a depth of up to 2 cm between autumn 2006 and summer 2007 according to methods explained by Kaushik (1987). The samples were cultured in solid nitrate free BG11 medium (Stanier *et al*., 1971), at constant irradiance ($2\mu E.m^{-2}s^{-1}$), pH 7.2, and constant temperature 27±2ºC. After colonization and isolation, samples were purified by several subculturings (Kaushik, 1987). Thallus growth, filament structure, and biometric frequency and abundance were determined using frequency symbols and procedures described by Quaseda and Valiente (1996). Briefly, planktonic cyanobacteria were sampled using 1-liter polyethylene wide- mouth jars. Benthic cyanobacteria were collected from 10-cm sediment cores. These cores were taken with a plexiglass cylinder, closing both extremes with rubber stoppers in order to avoid disturbance of the sediment-water interface. The water column was separated by pumping it out gently with a 100-ml syringe. The cores were then pushed from the top with a Teflon pestle, and the first 0.5 cm was cut with a razor blade. Cyanobacteria were enumerated by the dilution-plating technique. Each sample was homogenized with a blade and serial dilutions were made in distilled water. Aliquots of the dilutions were plated on BG11 lacking combined nitrogen and solidified with 1.5% (w/v) agar. Plates were incubated under 30 µmol photon $m^{-2}s^{-1}$at 28 ºC (+2ºC) for 3 weeks, and colonies were counted using a low magnification stereomicroscope and, when necessary, identified with an optical microscope. Pervious analysis had shown that under those conditions, no further colonies arose after incubation for an additional week. The results are expressed as colony forming units (CFU) per volume in water samples or per surface area in sediment samples (Gugger and Hoffmann, 2004). Colony formation and cell shapes were evaluated by light microscopy each day during a two weeks period. Phase contrast and epifluorescence microscopy were also performed on some occasions. The growth curves were attained using daily measurements of chlorophyll by the Jensen method (1978). Statistical analysis was done with software SPSS ver.10. Identification of samples was carried out by light microscopy and based on accepted criteria (John *et al*., 2002; Anagnostidis and Komárek, 1990; Prescott, 1962 and Desikachary, 1959). Photographs were taken with a Canon model camera attached to a Nikon microscope. Axenic condition was checked daily by microscopic observation.

Results

In this study eight species belonging to genera of *Calothrix*, *Gloeotrichia*, *Scytonema*, *Cylindrospermopsis*, *Cylindrospermum*, *Tolypothrix*, *Aulosira* were identified as new records for Golestan province and Iran. A liist of identified species is given in Table 1 and their photographs can be seen in Figure 5.

Description of the taxa

Nostocaceae

1- *Cylindrospermum licheniforme* (Bory) Kützing ex Bornet et Flahault, Ann. Sci. Nat. Bot., ser. 7, 7: 253. 1888.

Syn.: *Cylindrospermum spirale* Kützing

Thallus confluent; trichomes 3–4 *µm* broad, green, constricted at the cross walls. Cells quadrate, 3 *µm* long and broad. Heterocysts oblong, 5.5 *µm* long and 4 *µm* broad . Spores adjoining to the terminal heterocysts, oblong, 10 *µm* long and 6-7*µm* broad. Epispore smooth and brown.

General distribution: Europe, South-west Asia, E Asia, Australia and New Zealand.

Table 1. Algal flora and their frequencies on the Paddy-fields of Golestan province, NE Iran

Species	Spring					Summer					Autumn					Winter				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cylindrospermum lichenoforme	F	R	R	F	A	R	--	-	A	A	F	F-	R	--	--	R	R	F	F	F
	-	-	R	-	-	-		-	-	-	-		R			-	-	R	F	R
Cylindrospermum stagnale	F	-	R	F	-	-	R	f	-	-	-	F	R	R	F	R	-	-	-	R
	R	R	R	A	F	F	R	F	F	A	R	A	F	F	A	R	R	R	F	R
Aulosira implexa	-	-	R	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	A	F	F	R	F	F	A	A	F	F	A	R	F	A	R	R	F	A	F
Gloeotrichia longiarticulata	-	-	-	R	-	-	-	-	-	-	-	--	--	--	--	--	--	--	--	--
	A	A	F	F	R	F	F	R	R	R	-									
Calothrix ghosei	-	-	--	-	-	--	-	-	-	-	-	F-	R	F	F	-	-	--	-	-
	F	A		A	F		F	R	A	A	F		A	A	R	F	F		F	R
Sytonema multiramosum	-	-	-	R	F	-	-	-	R	-	R	F	R	F	R	-	-	-	R	-
	A	A	A	F	R	F	A	R	A	R	F	R	A	A	F	F	R	R	F	A
Tolypotrix conglutionata	R	-	-	R	-	R	-	-	-	-	R	-	-	R	-	-	R	-	-	R
	R	F	F	F	R	R	A	A	A	F	F	A	R	F	R	R	R	F	A	F
Cylindrospermopsis racibroskii	-	-	-	-	-	R	-	-	-	R	-	R-	--	R	R	--	--	--	--	--
	F	R	A	A	F	-	F	F	F	A	-			-	-					

Stations :1-Aliabad 2- Kordkoy 3- Minodasht 4- Azadshahr 5- Gorgan
D= Dominant (75-100%); A= Abundant (50-75%); F= Frequent (25-50%); R= Rare (<25%)

2- *Cylindrospermum stagnale* (Kützing) Bornet et Flahault, Ann. Sci. Nat. Bot., ser. 7, 7: 250. 1888.

Syn.: *Anabaena stagnalis* Kützing; *Cylindrospermum macrospermum* (Kützing) Bornet et Flahault

Thallus expanded, blue–green; trichome 4-5.5 *µm* broad and constricted at the cross walls. Cells nearly quadrate or cylindrical, 5 *µm* long and 3-3.5 *µm* broad. Heterocysts sub spherical or rectangular, 5*µm* broad and 6*µm* long. Spores oblong with rounded ends, 12 *µm* broad and 30 *µm* long, epispore smooth, brown outer layer.
General distribution: Europe, South-west Asia, E Asia, Australia and New Zealand.

3- *Aulosira implexa* Bornet & Flahault, Ann. Sci. Nat. Bot., ser. 7, 7: 257. 1888.
Filaments solitary or in clusters, long, straight or slightly curved, with firm, distinct brown sheaths enveloping one trichome. Trichomes not attenuated toward the ends, constricted at the cross wall, 11-12 *µm* broad. Vegetative cells are cylindrical or barrel-shaped, shorter than wide, 7-8 *µm* long, usually with prominent granules. Heterocysts cylindrical, solitary or sometimes in pairs, 7-8 *µm* long and14 *µm* broad ; akinetes usually spherical, oval to cylindrical, in rows between two heterocysts and towards heterocysts and sometimes irregular, 16 *µm* long and 8 *µm* broad.
General distribution: Mainly pantropical.

4- *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, Taxonomy and Biology of Blue-green Algae, 1. Int. Symp. Tax. Biol. Blue-green Algae, Madras, p. 55. 1972.
Syn.: *Anabaena* (*Anabaenopsis*) *raciborskii* Woloszyn-skaya; *Aphanizomenon kaufinannii* Schmidle; *Cylindrospermum droyphorum* Brubl & Biswas; *Anabaenopsis seriata* Prescott
Filaments solitary, straight, slightly curved, in several species narrowed toward the ends; without sheath; end cells conical or sharply pointed; vegetative cells cylindrical, 3 *µm* long and 5 *µm* broad,. Heterocysts terminal and intercalary, spherical, single with rounded end, at one or both ends of the trichomes, 3.5 *µm* long and 2.5*µ* broad. Akinetes ellipsoidal, rectangular, 9-10 *µm* long and 4 *µm* broad, in pairs or single, forming adjacent to or distant from the heterocysts.
General distribution: Pantropical and subtropical; occasionally in S. parts of temperate zone (warm seasons).

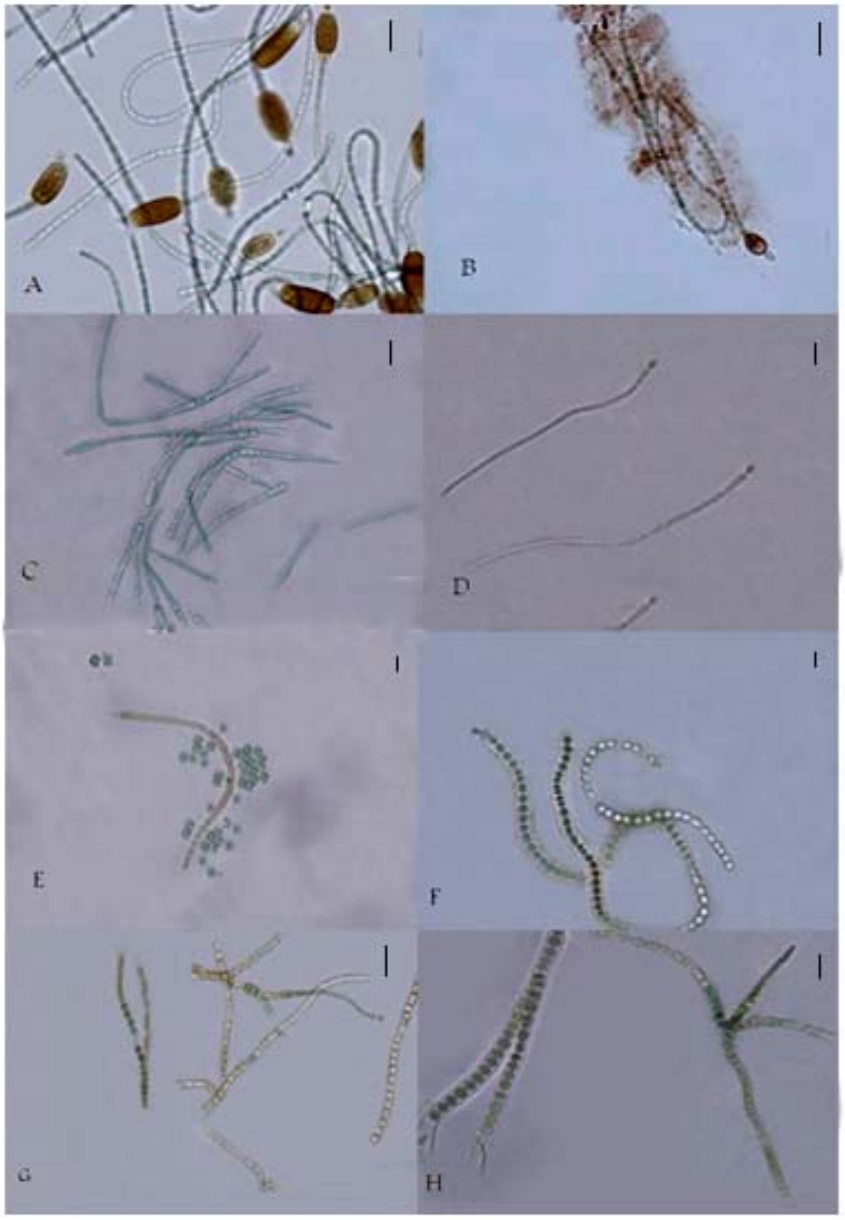

Fig. 1. **A-** *Cylindrospermum stagnale*, **B-** *Cylindrospermum licheniforme*, **C-** *Cylindrospermopsis raciborskii*, **D-** *Gloeothrichia longiarticulata*, **E-** *Calothrix ghosei*, **F-** *Aulosira implexa*, **G-** *Tolypotrix conglutinat*, **H-** *Scytonema multiramosum* (scale=10).

Rivulariaceae

1- *Calothrix ghosei* Bharadwja, The Myxophyceae of the United Provinces, India-I. Proc. Ind. Acad. Sci. B2: 99. 1935.

Filament nearly long, intricated, differentiated into basal and apical parts, solitary or small groups, rarely with false branch, 7 *µm* broad and in the middle about 6 *µm*; sheath always very thin, colorless; trichome green, unconstricted at the cross wall, funnel-like, widened at the ends. Cells cylindrical or barrel-shaped. Heterocysts basal, more or less semi-spherical, single, 4.5 *µm* broad. Spore solitary, jointed to basal heterocyst, cylindrical, 12-13 *µm* long, 5-6 *µm* broad.

General distribution: Asia.

2- *Gloeotrichia longiarticulata* G.S.West, Journ. Linn. Soc. Bot., 38: 183. 1907.
Trichomes with basal heterocyst, uniserial, attenuated especially near apical parts, constricted at the cross wall, more or less coiled, 8-9 *µm* broad. Vegetative cells cylindrical, rectangular, 3-3.5 *µm* long and 7-8 *µm* broad,. Sheath thin and hyaline. Heterocysts basal, spherical, ellipsoidal, single, 5-6 *µm* long and 4-5 *µm* broad,. Spores jointed to heterocysts, cylindrical, 4-5 *µm* long, 5-7*µm* broad.
General distribution: Africa.

Scytonemataceae
1- *Scytonema multiramosum* Gardner, Mem. New York Bot. Gard. 7: 81.1927.
Filaments free or in fascicles, sometimes densely coiled, commonly falsely branched with one or two lateral branches, 17 *µm* broad. Sheaths firm, hyaline. Trichome 10-12 *µm* broad, not diversified in basal and apical parts, uniserite, not constricted at the cross wall, usually with solitary, intercalary heterocysts. Cells olive-green in some species with granular content, sub-spherical or sub-quadrate, 9-10 *µm* long, 7-8 *µm* broad.
General distribution: America.

2- *Tolypothrix conglutinate* Borzi var. *colorata* Ghose, Ghose, Jour. Linn. Soc. Bot. 46: 345.
Filaments blue-green, straight; basal parts with heterocysts, commonly falsely branched, usually with solitary lateral branches; trichomes 7 *µm* broad, in some parts constricted at the cross wall. Sheath thin, 0.7-1 *µm* broad. Trichomes and branches uniseriate, with one basal heterocysts, cylindrical to barrel shaped, 7-8 *µm* long and 4 *µm* broad.
General distribution: India, Pakistan.

Discussion
Collectively, our knowledge about cyanobacteria of Golestan Province is limited. A few reports have been published, and greatest consideration has been given to Nostocalean species (Norouzi *et al.*, 2004; Siahbalaie *et al.*, 2008; Shariatmadari and Riahi, 2010). Temperature, pH, and salt stress are among the most important factors that affect the growth and productivity of microorganisms (Whitton and Potts, 2000).
Lower acidities and high electrical conductivity (EC) may simply have a delaying effect on the development of the algal flora (Broady, 1984). The pH in different stations of Golestan Province is between 7-8 and EC is 0.5-1.2 (µs/cm^{-1}). These parameters seem to be suitable for growth of cyanobacteria (Shokravi *et al.*, 2007; Siahbalaei *et al.*, 2010).
In our study, cyanobacteria constituted 75% of the algal flora in the paddy-fields of Golestan Province. The algal flora on the paddy-fields, agriculture lands, and rivers consisted of planktonic, epipelic flora and attached species of algae. Periphytic species belonging to *Gloeotrichia longiarticulata*, were the most common species identified. *Tolypothrix conglutinata*, *Aulosira implexa* ,*Scytonema multiramosum*, *Calothrix ghosei*, *Cylindrospermum stangnale* , *Cylindrospermum licheniforme* which are epipelic,epilithic and epidaphic,and *Cylindrospermopsis raciborskii* which is planktonic were also common. Many algal species such as *Cylindrospermum stagnale*, *Aulosira implexa*, *Scytonema multiramosum* which are useful indicators in paddy-fields were observed in our study, but *Calothrix javanica*, *Anabaenopsis tanganyikae* were not observed.
The number of species at each site ranged from 7-8 with a maximum at Azadshahr, Gorgan and a minimum at Minodasht, Kordkoy and Aliabad. Dominant species in all stations and in all seasons were *Cylindrospermum stagnale*, *Aulosira implexa*, *Scytonema multiramosum*, and *Tolypothrix conglutinata*. *Gloeotrichia longiarticulata* and *Cylindrospermopsis raciborskii* were generally found during spring and summer.
The species compositions in the Gorgan and Azadshahr stations were similar, but the number of species in Azadshar was higher than Gorgan. Undoubtedly, no single factor determined the development of algal flora, rather all these factors combined affected algal development. The similarity in the species composition of the flora in the various paddy-fields was due to the similarity in pH (7.2-8.2), EC (0.51-1.5 ms/cm^{-1}), temperature (30-32°C), and depth of sample collection. On the other hand a complication was

observed between the algal flora of planktonic and benthic. The results of this study contribute to our knowledge of heterocystous cyanobacteria in the paddy- fields of Iran. Broadness and length of filaments, trichome structure, heterocyst form and position and spores position are important characteristics for identification of heterocystous cyanobacteria in Iran. Results of this survey evidence high morphological variation among some cyanophyta such as *Cylindrospermum*, *Calothrix*, *Scytonema* in paddy fields. Better identification keys are needed for correct identification of the cyanobacteria in the studied area. Nevertheless, our results present a preliminary picture of the morphological and taxonomical situation of cyanobacteria in paddy fields of north of Iran.

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