NEW MORPHOSPECIES OF HETEROCYSTOUS CYANOPHYTA FROM PADDY-FIELDS OF GOLESTAN PROVINCE, IRAN

R. Siahbalaei, S. Afsharzadeh, & Sh. Shokravi

Received 08.07.2012. Accepted for publication 07.11.2012.

Siahbalaei, R., Afsharzadeh, S. & Shokravi, Sh. 31 12 2012: New morphospecies of heterocystous cyanophyta from paddy fields of Golestan province, Iran -*Iran. J. Bot. 18 (2): 311-317* . Tehran.

The algal flora of 5 stations from paddy-fields of Golestan province was investigated between October 2006 and September 2007. In this study 10 interesting filamentous heterocystous Nostocacean morphospecies, recorded for paddy fields of Iran. Among these taxa *Nostoc verrucosum* Voucher ex Born.et Flah, *N. comminutum* Kützing *N. hatei* Dixit, *Anabaena mendotae* Trelease, *A. aphanizomenoides* Fortit, *Trichormus anomalus* (F. E. Fritsch) Komarek & Anagnostidis, *T. fertilisimus* (C. B. Rao) Komarek & Anagnostidis, are reported as seven new records from Iran. These taxa described based on their morphology and ecology.

Roghayeh Siahbalaei (correspondence <m.siahbala@gmail.com>), Saeid Afsharzadeh , Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran. Shadman Shokravi, Department Biology, Islamic Azad university-Branch Gorgan, Gorgan, Iran.

Key words. Cyanbacteria, Golestan province, Iran, morphospecies, new records, rice fields.

گونه‌های مورفولوژیکی جدید از سیانوفیتای هتروسیست‌دار در شالیزارهای استان گلستان

رقیه سیاه بالایی، دانش آموخته کارشناسی ارشد، گروه زیست‌شناسی دانشگاه اصفهان.

سعید افشارزاده، استادیار گروه زیست‌شناسی دانشگاه اصفهان.

شادمان شکروی، استادیار گروه زیست‌شناسی دانشگاه آزاد اسلامی واحد گرگان.

فلور جلبکی از ۵ ایستگاه از شالیزارهای مورد مطالعه در مهر ماه۱۳۸٦ تا مرداد ۱۳۸۷ مورد بررسی قرار گرفت. در این مطالعه ۷ گونه مورفولوژیک از جلبکهای هتروسیست دار برای ایران گزارش می‌گردد. این تاکسون‌ها *Nostoc verrucosum* Voucher ex Born. et Flah, *N. comminutum* Kützing *N. hatei* Dixit, *Anabaena mendotae* Trelease, *A. aphanizomenoides* Fortit, *Trichormus anomalus* (F. E. Fritsch) Komarek & Anagnostidis, *T. fertilisim*us (C. B. Rao) Komarek & Anagnostidis به عنوان ۷ گونه‌ی جدید از شالیزارهای ایران گزارش می‌گردند. ویژگیهای آرایه‌شناسی این گونه‌ها و اطلاعات پیرامون پراکنش اکولوژیکی آنها نیز ارائه می‌گردد.

INTRODUCTION

The Cyanophyta are a morphologically distinct group of oxygenic photosynthetic organisms that inhabit in terrestrial and aquatic ecosystems (Prasanna et al. 2008). They are used as fertilizers, soil conditioners and are a source of livestock feed (Anagnostidis & Komarek 1990). Taxonomic classification is a method for understanding and reviewing of diversity. However, a combination of traditional and modern taxonomy (in addition of physiology and biochemistry) are needed to determine the real place of the genera *Nostoc* and *Anabaena* and stigonematalean cyanophyta as a whole. On the other hand, descriptions of evidently new morphospecies and ecospecies are very important for recognition of Cyanophyta diversity under natural conditions.

Approximately 534 species and infra species of *Anabaena* and 334 of *Nostoc* species and infra species has been identified. (www.algaebase.org).

The paddy-fields of Golestan provice were containing nearly 65000 hectares of total cultivation fields in last year and its ecosystem provides a favorable environment for the growth of blue- green algae with respect to their requirements for light, water, high temperature and nutrient availability. This could be the reason that blue-green algae grow in higher abundance in paddy-fields (Grossman et al. 1993). Up to now a few species of Cyanophyta has been recorded

Table 1: Algal flora, *Nostoc*, *Anabaena* and *Trichormus* and their frequency symbols on the Paddy-fields of Golestan province.

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
N. paludosum	F	R	F	F	D	F	-	F	F	-	F	A	F	F	A	A	F	R	R	A
N. comminutum	R	-	-	R	F	R	-	R	F	R	F	R	R	-	R	R	-	R	-	R
N. verrucosum	F	-	-	R	F	-	-	-	-	-	-	R	-	-	-	-	-	R	-	-
N. hatei	R	-	-	R	F	-	-	-	R	-	-	-	R	-	-	-	-	R	-	-
A. sphaerica	F	R	R	R	F	R	-	-	-	-	-	-	-	R	-	R	R	-	-	-
A. aphanizomenoides	F	R	F	-	F	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-
A. mendotae	R	R	-	-	R	-	-	R	R	-	A	F	F	A	A	F	F	F	F	A
A. spiroids	F	-	-	F	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. fertilissimus	A	A	F	A	A	F	F	F	R	F	A	R	R	A	D	A	F	A	A	A
T. anomalus	-	-	-	R	R	-	-	-	-	-	F	R	R	-	F	-	-	-	-	-

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

from paddy fields of Iran (Siahbalaie & al. 2008 and 2010, Shokravi & al. 2007, Sepehri & al., 2003). The aim of this paper is to contribute to the knowledge on the algal flora of paddy-fields based on morphological investigations of the Cyanophyta of the paddy-fields in Golesten province.

MATERIALS AND METHODS

Five stations were chosen in different areas of the paddy-fields. The samples were taken from the surface to 2 cm depth of non flooded soils between autumn 2006 and summer 2007 (Kaushik 1987). The samples cultured in solid BG_{110} medium (NaCL , 17.65 mM , mg So4 . 7H2O, 0/3mM , cacl2 , 2H2O , 0/25 mM , K2HPo4.3 H20,0/18Mm,Na2 Mg EDTA , 0/003 mM , citrate ferric , 0/02 mM Acid citric , 0/029 mM , Na2Co3 , 0/188 mM , microelements lml 1)-1. The cultivation was done at constant irradiance ($2\mu E.m^{-2}.s^{-1}$), pH: 7.2 and temperature 25 ± 2^0C. After colonization and isolation, samples were purified by several sub culturing. (Kaushik, 1987). Frequency and abundance have been determined using procedure that has been described by Quaseda & Valiente (1996).

Briefly planktonic Cyanobacteria were sampled with 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 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 30umol photon m-2s-at 28 0C (+2OC) for 3 weeks, and colonies were counted using a low magnification stereomicroscope. Pervious analysis showed that under those conditions no further colonies were detectable with incubation for an additional week. The results are expressed as colony forming units (CFU) per volume in water samples or per surface in sediment samples. Identification of samples was carried out by light microscopy and based on John et al. (2002), Anagnostidis & Komarek (1990), Prescott (1962) and Desikachary (1959).

RESULT

In the present study 4 species belonging to the genus *Nostoc*, 4 species belonging to the genus *Anabaena* and 2 species belonging to the genus *Trichormus* were identified. The species, their photographs and their distribution on the paddy-fields of Golestan is listed in tables 1 & 2 and figures1, 2, 3, 4.

Cyanophyta
Cyanophyceae
Nostocales
Nostocaceae

Nostoc verrucosum Vaucher ex Bornet & Flahault
Nostoc comminutum Kützing
Nostoc hatei S. C. Dixit
Nostoc paludosum Kützing ex Bornet & Flahault
Anabaena sphaerica Bornet & Flahault
Anabaena spiroides Klebahn
Anabaena mendotae W.Trelease
Anabaena aphanizomenoides Fortit
Trichormus anomalus (F. E. Fritsch) Komárek &

Table 2: Comparison of phenotypic characters of *Nostoc*, *Anabaena* and *Trichormus.*

Species	width of Trichome (*μm*)	sheaths	cell length (*μm*)	shape of cell	shape of heterocyst	position of heterocyst	shape of akinetes	position of akinetes to heterocyst
N. Verruscosum	7-8	thick	*6.5*	elliptical-cylindrical	spherical	basal	cylindrical	spore more or less like vegetative cell, but slightly cylindrical
N. comminutum	4	-	*3.7*	spherical-square	elliptical	intercalary-basal	spherical	irregularly
N. hatei	*4-5*	-	4-4.5	square	spherical-elliptical	basal	basal square	spore more or less like vegetative cell
N. paludosum	4	Thick	*3*	spherical	elliptical	intercalary	spherical -oval	irregularly
A. spharica	3	thin-colorless	2.5	barrel-shaped, square	spherical-elliptical	intercalary-basal	sub spherical-oval	one or both sides of heterocyst
A. spiroids	6-7	fine-colorless	6	spherical	spherical	basal	spherical-elliptical	Distant from heterocytes
A. mendotae	3	-	2.5-3	square	spherical	intercalary-basal	elliptical	one side or distant from heterocyst
A. aphanizomenoides	4-5	-	3-3.5	spherical-square	spherical	intercalary	elliptical	both sides of heterocyst
T. anomalus	3-3.5	fine-colorless	3	rectangular	Solitary-spherical	intercalary	rectangular	irregularly
T. fertilissimus	5.5-6	firm	5	spherical-elliptical	Sub spherical	intercalary-basal	spherical- in rows	in rows between or one side of heterocyst

Anagnostidis
Trichormus fertilisimus (Rao, C. B) Komárek & Anagnostidis

DISCUSSION

The diversity of organisms (both in nature and in culture) can be reviewed and characterized only by help of the taxonomic classification. Our data will help in an identification of Cyanophyta morphospecies, especially *Nostoc* and *Anabaena* genera.

The intra generic taxonomy of the genera *Nostoc* and *Anabaena* is very complicated (Turner, 1997, Rudi et al. 1998). In this study, we try to use common manuals for determination with regard to special morphological variations of specimens with emphasize on local conditions, seven of the morphospecies are first records for Iran and show that morphological variation of some Cyanophyta such as *Nostoc* and *Anabaena* are very high, that need to improve identification keys (Shokravi, et al. 2002). Cyanophyta in the algal flora of paddy-fields formed the majority with a ratio of 75 %, this can be explained by the variety of habitats or may have been the result of physical, chemical or geographical differences between paddy fields (Siahbalaei et al. 2010). The flora of algae in paddy-fields varied between stations and seasons. The number of species at each site ranged from 1 to 10 with Maximum at Gorgan and Aliabad stations, and minimum at Kordkoy station. Variation changes during the season and forms characteristic communities in climax stages. In spring (April and May), it was observed that diatoms and uni-cellular green algae were dispersed along the water, while the Cyanobacteria had lesser frequency. *Anabaena mendotae, Trichormus fertilissimus*, *Nostoc comminutum* and *N. paludosum* were dominant species in all stations and seasons. *Anabaena aphanizomenoides*, *A. spiroides* and *Trichormus anomalus* were not observed in summer and winter.

Collectively our knowledge about Cyanophyta of Golestan province is limited until now; a few reports have been published with the highest degree of consideration from lakes, rivers and soils of Iran. *Anabaena catenula* (Ramzannejad-Ghadi 2008), *A.*

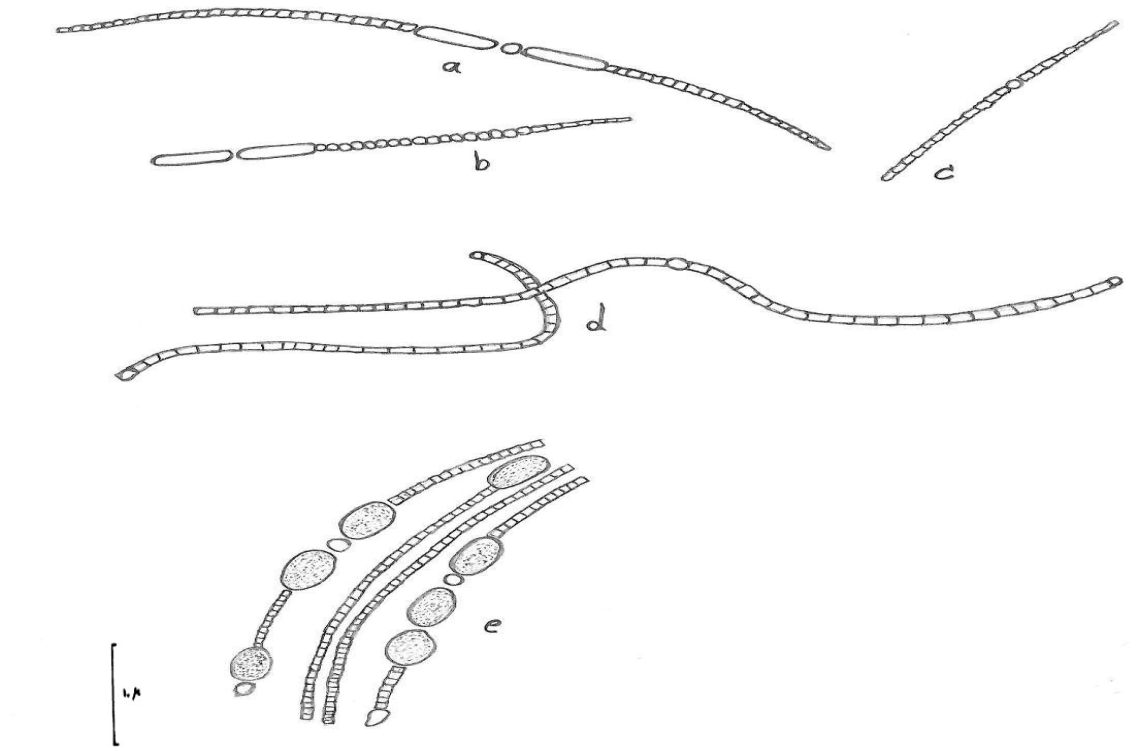

Fig.1: a,b,c, *Anabaena aphanizomenoides*, d: *Trichormus anomalus*; e: *A. spharica*.

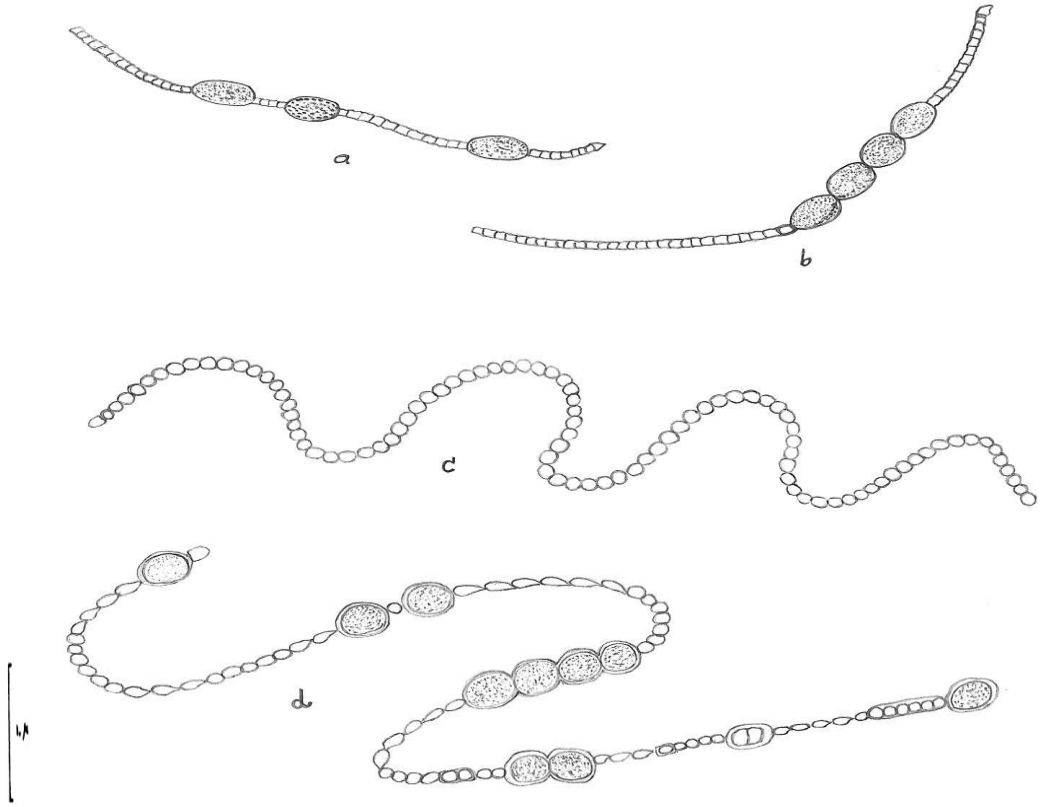

Fig. 2: a,b: *Anabaena mendotae*, c: *A. spiroids*, d: *Trichormus fertilissimus*.

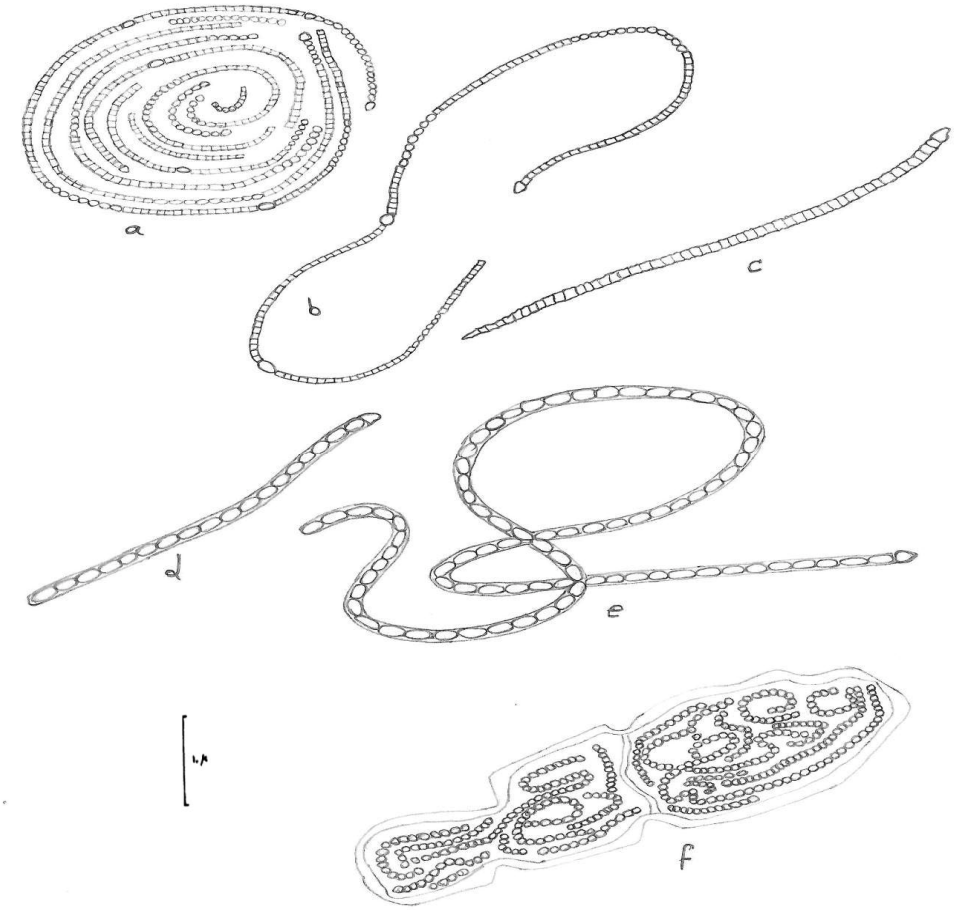

Fig. 3: a,b, *Nostoc comminutum*, c: *N. hatei* , d,e: *N. verruscosum* , f: *N. paludosum*.

planctonica (Bagheri & Ramzannejad-Ghadi 2008), *A. caspica, A. azolla*, *Nostoc pruniforme* (Zarei-darki, 2009), *Anabaena spiroides* (Saadatnia & Riahi, 2009), *Anabaena torulosa*, *A. variabilis*, *A. oscillarioides*, *Nostoc spongiforme*, *N. ellipsosporume*, *N. commune* (Nowruzi & Ahmadi-Moghadam, 2007), *Nostoc oryzae*, *N. calcicola* and *Anabaena vaginicola* (Shariatmadari & Riahi 2010). In this study some characteristics such as dimensions of vegetative cells, dimensions and shape of heterocysts, akinetes and position of heterocyst with regard to akinetes especially in *Anabaena* are noticeable characters for identification of *Nostoc* and *Anabaena* species.

REFERENCES

Anagnostidis, K. & Komarek, J. 1990: Modern approaches to the classification of Cyanobacteria, Stigonematales. -Archieves for Hydrobiology, 14: 224–286.

Bagheri, L. & Ramzannejad-Ghadi, R. 2008: Epiphytic algae of Miankaleh International Wetland. 1st National Congress on Fishery Resources of Caspian Sea. 18-19 Nov. -Gorgan University, Agricultural Sciences and Natural Resources.

Desikachary, T. V. 1959: Cyanophyta. Indian Council of Agricultural Research, monographs on Algae. -New Delhi, India.

Grossman, A. R., Schaefer, M. R, Chiang, G. G. & Collier, J. L. 1993: Environmental effects on the light-harvesting complex of Cyanophyta. -Journal of Bacteriology 175 (3): 575-582.

John, D. M., Whitton, B. W. & Brook, A. J. 2002: The Freshwater Algal Flora of the British Isles. -Cambridge University Press.

Kaushik, B. D. 1987: Laboratory methods for blue–green algae. -Associated Publishing Company, New Delhi, India.

Nowruzi, B. & Ahmadi-Moghadam, A. 2007: New records of relationship between soil macroelements and the distribution of heterocystous Cyanobacteria in paddy field, wheat fields and wood in Golestan province. -Iranian Journal of Biology, 20: 89-98.

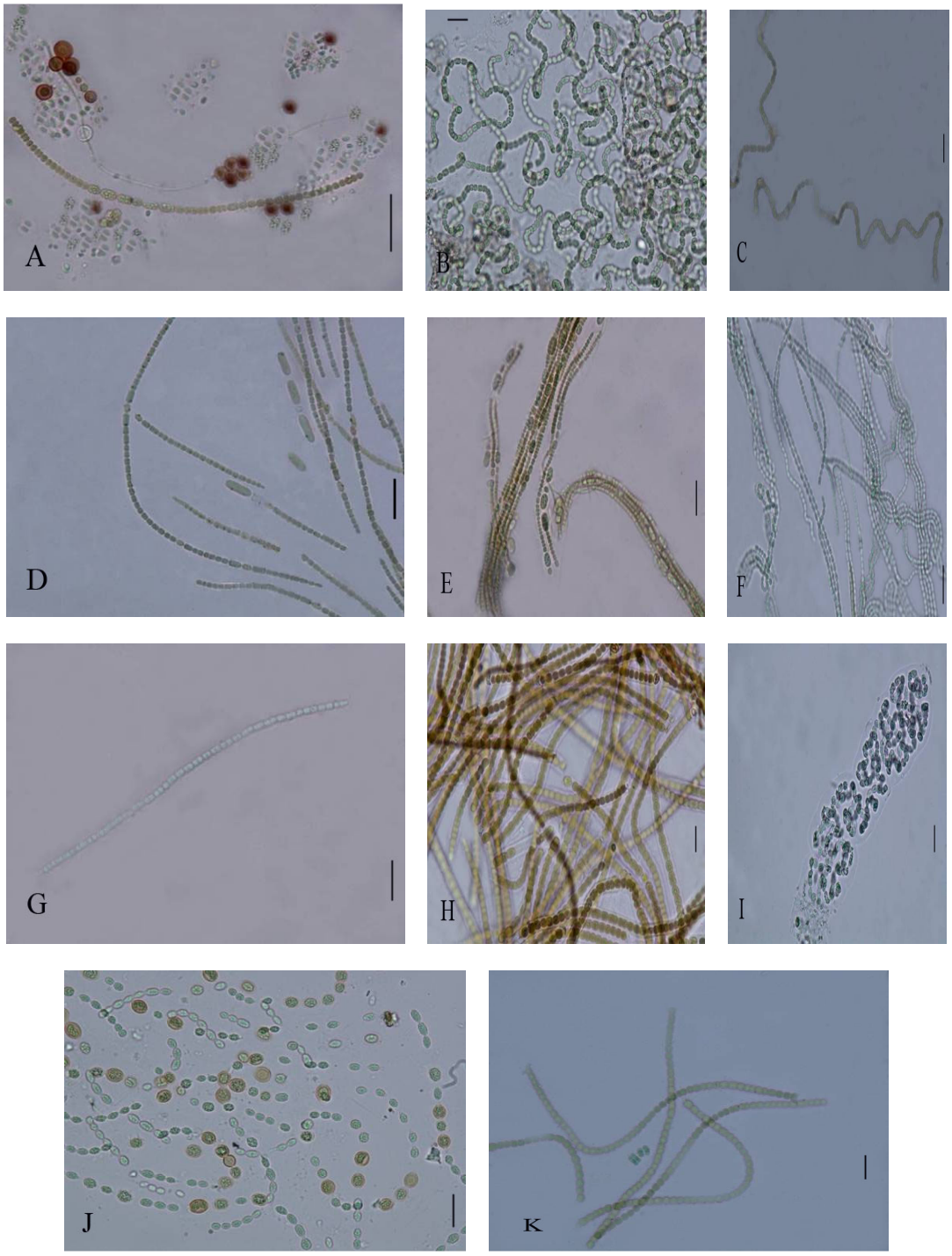

Fig. 4: a: *Anabaena mendotae*, b,c: *A. spiroids*, d: *A. aphanizomenoides*, e: *A. sphaerica*, f: *Nostoc comminutum*, g: *N. hatei*, h: *N. verruscosum*, i: *N. paludosum*, j: *Trichormus fertilissimus*, k:*T. anomalus*.

sustainability- promises and challenges. -Indian J. Microbiol. 48:89-94.

Prasanna, R., Jaswal, P. & Kaushik, B. D. 2008: Cyanobacteria potential options for environmental Prescott, G. W. 1962: Algae of the Western Great Lake Area. -W.M.C. Brown Company Pub.

Queseda, A. & Valiente, E. F. 1996: Relationships between abundance of Cyanobacteria and environmental features in Spanish rice-fields. -Microbial Ecology 32:59-71.

Ramzannejad-Ghadi, R. 2008: Epipelic algae of Miankaleh International wetland (North of Iran).1st National congress on fishery resources of Caspian Sea .18-19 Nov. -Gorgan University, Agricultural Sciences and Natural Resources.

Rudi, K., Skulberg, O. M. & Jakobsen, K. S. 1998: Evolution of Cyanobacteria by exchange of genetic material among phyletically related strains. -Journal of Bacteriology 180: 3453-3461.

Saadatnia, B., & Riahi, H. 2009: Plant Soil environment 55 (5): 207-212.

Sepehri, S., Nejad-Satari, T., 2003: Studies on blue-green algae (Cyanophyta) with emphasis on Stigonemataceae family from paddy fields around Gorgan. -Dissertation of Master degree. Azad University, Tehran, Iran.

Shariatmadari, Z. & Riahi, H. 2010: New records of heterocystous Cyanophyta from paddy fields of Iran. –Rostaniha 11 (2): 113-119.

Siahbalaei, R., Afsharzadeh, S., & Shokravi, Sh. 2008: Three new records of Oscillatorian cyanophyta for the paddy fields algal flora of Iran. -Journal on Plant Researches 1 (9):1-5

Siahbalaei, R., Afsharzadeh, S., Shokravi, Sh. & Nekouei, Sh. 2010: Some new records of Oscillatorian Cyanophyta from paddy fields of Golesten province. -Iranian Journal of Botany, Tehran., Iran 16 (2): 314-319.

Shokravi, Sh., Soltani, N. & Baftechi, L. 2007: Cyanobacteriology. -Islamic Azad Univ. Publication.

Shokravi, Sh. 2002: Applied research management of cyanobacteria in Iran: problems and solutions. -The first Iranian Congress on Applied Biology, Islamic Azad University, Mashhad, Iran.

Turner, S.1997: Molecular systematic of oxygenic photosynthetic bacteria. -Plant Systematic and Evolution 11:13-52

www.algaebase.org

Zarei-Darki, B. 2009: Marine species in the algal flora of the Anzali swamp (Iran). -Russian Journal of Marine Biology.vol. 35, no3: 200-205.