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Taxonomic survey of cyanobacteria of Urmia Lake (NW Iran) and their adjacent ecosystems based on morphological and molecular methods

بررسی تاکسونومیکی سیانوباکتری‌های دریاچه ارومیه و اکوسیستم‌های مجاور آن‌ها براساس روش‌های مولکولی و مورفولوژیکی*

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Abstract

In the present study, the cyanobacterial flora of Urmia Lake (NW Iran), one of the largest salt lakes in the world and adjacent rivers is investigated. Urmia Lake comprises different micro-organisms including cyanobacteria which have a major role in aquatic ecosystems. Cyanobacterial samples were collected in 2008–09 from Urmia Lake and its surrounding ecosystems. All samples were cultivated, isolated and identified using morphological keys basically and also molecular methods via 16S rRNA sequencing in some cases. Following cyanobacterial species were isolated from Urmia Lake: *Spirulina maxima*, *Gloeocapsa* sp., *Oscillatoria* sp., *Nodularia spumigena*, *Phormidium fragile*, *Oscillatoria salina*, *Plectonema notatum*, *Aphanocapsa grevillei*, *Trichormus variabilis*, *Lyngbya* sp., *Phormidium* sp., *Microcoleus chthonoplastes*, *Leptolyngbya* sp., *Chroococcus turgidus*, *Microcoleus* sp., *Chroococcus dispersus*, *Nodularia* sp. and *Nostoc* sp.. These variations may be referred to limited sampling, or increased salinity of the lake during recent years that has eliminated some non-tolerant species. This study reflects the biologically activation of Urmia Lake despite the hypersalinity and extreme condition of it.

Keywords: Cyanobactertia, hypersaline lake, taxonomic survey, Urmia Lake, 16S rRNA

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خلاصه

در تحقیق حاضر، فلور سیانوباکتریایی دریاچه ارومیه، یکی از بزرگترین دریاچه‌های نمکی جهان و رودخانه‌های اطراف مورد بررسی قرار گرفت. دریاچه ارومیه متشکل از میکروارگانیسم‌های مختلف شامل سیانوباکتری‌ها است که نقشی اساسی در اکوسیستم‌های آبی دارند. نمونه‌های سیانوباکتریایی در سالهای ۸۸-۱۳۸۷ از دریاچه ارومیه و اکوسیستم‌های اطراف جمع‌آوری گردید. تمام نمونه‌ها کشت شده، جداسازی شده و با استفاده از کلیدهای مورفولوژیکی به طور اولیه و نیز با روش‌های مولکولی (16S rRNA) در تعدادی از موارد، مورد شناسایی قرار گرفتند. سیانوباکتری‌های زیر از دریاچه ارومیه جدا شدند: *Oscillatoria* sp., *Gloeocapsa* sp., *Spirulina maxima*, *Nodularia spumigena*, *Phormidium fragile*, *Oscillatoria salina*, *Plectonema notatum*, *Aphanocapsa grevillei*, *Trichormus variabilis*, *Lyngbya* sp., *Phormidium* sp., *Microcoleus chthonoplastes*, *Leptolyngbya* sp., *Chroococcus turgidus*, *Microcoleus* sp., *Chroococcus dispersus*, *Nodularia* sp. و *Nostoc* sp. این تنوع را می‌توان به نمونه-برداری‌های محدود یا افزایش شوری دریاچه در طول سالیان اخیر نسبت داد که باعث حذف تعدادی از نمونه‌های غیرمقاوم شده است. این مطالعه حاکی از فعالیت بیولوژیکی دریاچه ارومیه علیرغم شرایط افراطی و شوری فوق اشباع آن دارد.

واژه‌های کلیدی: بررسی تاکسونومیک، دریاچه ارومیه، دریاچه فوق اشباع از نمک، سیانوباکتری‌ها، 16S rRNA

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Introduction

Urmia Lake, $37^0 30^/$ N $46^0 0^/$ E, one of the largest salt lakes in the world is located at northwestern of Iran at an altitude of 1250 m above sea level. Urmia Lake is an oligotrophic lake of thalassohaline origin exposed to the annual hydrological regime with flooding and desiccation periods (Ghaheri & Baghal-Vayjooee 1999). The brackish to hypersaline habitats showed salinity gradients mostly 217–235 gL^{-1} (NaCl) to saturation (>300 gL^{-1}). Annual inflow into the lake is 106–6,900 m^3, originated from rivers and flood waters. Most rivers enter from the south. Underground springs are also a source of some water. The total catchment area of the lake is 51,440 km^2 and contains 21 permanent and seasonal rivers and 39 episodic ones mostly from south. There are no surface outflows from the lake. The largest river is Zarineh River, with a total annual discharge value of 2–109 m^3 (Eimanifar & Mohebbi 2007, Mohebbi *et al.* 2006).

Extreme environments such as saline and hypersaline lagoons are hostile to most forms of life, however, they harbor significant populations of microorganisms. Their colonization by primary producers (algae and cyanobacteria) demonstrates that such organisms can adapt to extreme ecological niches. Both eukaryotes and prokaryotes have evolved a broad variety of adaptations, including the accumulation of osmolytes, to cope with osmotic and ionic stress (Kirst 1995, Oren 2000). The cyanobacteria are a diverse and cosmopolitan bacterial phylum and possess a number of unique biological characteristics in an array of habitats (Andrea *et al.* 2008). The classification of organisms has traditionally been based on similarities in their morphological, developmental and nutritional characteristics. The main problems in applying morphological criteria in cyanobacterial classification arise from the flexibility of morphological features which depends on environmental conditions. Sometimes microscopy and enrichment cultures have limited uses since distinct species of cyanobacteria can share similar simple morphological and cultivation limitations.

It is now clear that, however, cyanobacterial classification based on these criteria does not necessarily correlate with natural (i.e. evolutionary) relationships, as defined by macromolecular sequence comparisons (Stackebrandt & Woese 1981). A much broader application of molecular phylogenetic analysis to the description of microbes, both eukaryotic and prokaryotic, seems desirable. All of the available molecular methods for evaluating phylogenetic relationships (e.g. DNA-DNA and DNA-rRNA hybridization, 5S rRNA and protein sequencing, 16S rRNA oligonucleotide cataloging, enzymological patterning etc.) have advantages and limitations. In general, macromolecular sequences seem preferred because they permit quantitative inference of relationships. The approach described here rapidly provides partial sequences of 16S rRNA that are useful for phylogenetic analysis (Lane *et al.* 1985). The development of techniques for the analysis of 16S rRNA sequences in natural samples has already greatly enhanced detection and identification of cyanobacteria in nature (Norris *et al.* 2002). It should also be noted that in studies where 16S rRNA partial gene sequences have been used, conflicts between morphological and molecular identification of some cyanobacterial sequences have been found (Hongmei *et al.* 2005). On the other hand, new methods of phylogenetic inference such as 16S rRNA (gene) sequence analysis have shown that morphological characters may or may not result in a phylogenetically reliable taxonomy among cyanobacteria. Studies using 16S rRNA gene data from environmental samples and cultures have demonstrated that genotypic diversity can far exceed phenotypic diversity estimated by observation and culture techniques (Soltani *et al.* 2010).

The studies on cyanobacteria of Iran are mostly around physiological or multi-discipliner approaches and taxonomic surveys are restricted (Mohebi *et al.* 2006, Riahi *et al.* 1994, Soltani *et al.* 2007, Soltani *et al.* 2010). Riahi *et al.* (1994) surveyed restrictively the algal flora of Urmia Lake which indicated six cyanobacterial species. In this research, we report the results of a

polyphasic study of the cyanobacterial communities in Urmia Lake in Iran. Morphological identification of cyanobacteria carried out by related keys (Prescott 1970, Smith 1950). We combined the use of environmental 16S rRNA gene analysis and microscopic examination of culture isolates to characterize cyanobacterial diversity in adjacent ecosystems in Urmia Lake.

Materials and Methods

Cyanobacterial samples were collected from five sites in Rahmanloo Port, Golmankhaneh Port, Zarineh River, Nazloochia and Talkheh Rivers (Table 1).

Table 1. Sites description and characteristics

Site No.	Locality	Location co-ordinates	Height (m)
1	Rahmanloo Port (BR)	N 37° 33′ 39″ E 45° 43′ 29″	1305
2	Golmankhaneh Port (BG)	N 37° 36′ 71″ E 45° 14′ 21″	1253
3	Zarineh River (ZR)	N 37° 09′ 55″ E 45° 56′ 37″	1291
4	Nazloochia ana (NC)	N 37° 43′ 12″ E 45° 13′ 41″	1261
5	Talkheh River (TR)	N 37° 51′ 13″ E 45° 48′ 20″	1247

- Growth media, isolation and culture

Microalgae were cultured in freshwater medium, BG11 (Rippka, 1988). containing $NaNO_3$ 1.5 gL^{-1}, K_2HpO_4 0.04 gL^{-1}, $CaCl_2.2H_2O$ 0.036 gL^{-1}, $MgSO_4.7H_2O$ 0.075 gL^{-1}, $FeSO_4$ 0.006 gL^{-1} EDTA 0.001 gL^{-1}, Na_2CO_3 0.02 gL^{-1}, Citric acid 0.036 gL^{-1}, agar 10 gL^{-1} and 1 mL L^{-1} of a Trace metal mix A_5 containing H_3BO_3 2.58 gL^{-1}, $MnCl_2.4H_2O$ 1.18 gL^{-1}, $ZnSO_4.6H_2O$ 0.222 gL^{-1}, $NaMoO_4$ $.2H_2O$ 0.36 gL^{-1}, $CuSO_4.5H_2O$ 0.075 gL^{-1} and $Co(NO_3)_2.6H_2O$ 49.4 mg L^{-1} .Solid media were prepared using agar to 1% (w/v) before autoclaving. The incubation took place at 26° C under continuous illumination of 500 to 900 Lux prepared by cool white fluorescent tubes. Various approaches have been taken to try to develop more efficient methods to purify cyanobacteria. These have included mechanical separation of the cyanobacteria by micromanipulation, differential filtration and repeated transfer of cells (Garcia-Pichel *et al.* 1998). Variation of cyanobacteria was restricted to a relatively few representatives due to extreme conditions of habitats.

Gliding cyanobacterial strains were purified by their phototactic movement on scored agar. *Oscillatoria* strains were purified on BG-11 agar supplemented with 0.1% (w/v) $Na_2S \times 9H_2O$ (pH approximately 10), which stimulated their gliding movement. Other strains were purified on ordinary BG-11 agar. The plates were incubated at 26° C under unidirectional light, the scores parallel with incident light (50 to 100 lx) (Vaara *et al.* 1979). Parallel subsamples of soil (10 g) and pool sediment (10 mL) were aseptically suspended in 75 mL of sterile liquid medium or directly plated (1 g soil or benthic mat, 1 mL pool-water) on 1% agar plates with the same medium composed of BG-11.

- Morphological classification

Morphological identification of cultivated cyanobacteria was based on characters observable under a light microscope (400–1,000×), using an Olympus BH2 compound microscope and camera systems. The key features taken into consideration for the identification and differentiation isolates were: the habitat, the colony shape, the arrangement of filaments, morphology of sheaths, trichome and cell shapes, differentiated cells (heterocysts and akinetes) (Smith 1950, Prescott 1970, Kaushik 1987, John *et al.* 2003).

- DNA extraction

After centrifugation of the samples, cells were harvested by centrifugation at 14000 g for 20 min. at room temperature, washed three times with 1.2% (w/v) NaCl for exopolysaccharide removal and frozen at –20° C until use. To obtain cell lysis, a frozen pellet (about 1.5 g) was suspended in TN buffer (10 mM Tris/HCl, 100 mM NaCl) at pH 8 and incubated with 100 mg proteinase K and 5 mg lysozyme for 60 min. at 33° C under slow shaking (Marco *et al*. 1999). Extraction of DNA was carried out with Bio NEER Kit and Gel extraction.

- PCR amplification of cyanobacterial 16S rRNA

Primer design was based on an alignment of all 16S rRNA sequences from cyanobacteria. The 16S rRNA gene was amplified by using the PCR conditions described below and primers CYF 359 (5′-GGA ATY TTC CGC AAT GGG-3′) and CYR 781(5′-GAC WGG GGT TAA TCC C-3′) (Nübel *et al*. 1997). The reaction was performed in a final volume of 100 µl by BioERLittle Genuis PCR System, using the following amplification mixture. The temperature program was composed then 30 cycles of 30 sec. denaturation at 94° C, 30 sec. annealing at 48° C, 1 min., 30 sec. extension at 72° C. All reactions were repeated at least twice, always including both negative (DNA free) and positive controls. The successful amplifcation of the expected fragment (1500 bp) was checked by electrophoresis in 1% (w/v) agarose gel at 210 V cm^{-1} for 35 min. in TBE buffer (89 mM Tris borate, 89 mM boric acid, 2 mM EDTA; pH 8), stained with ethidium bromide 0.4 mg and observed under UV light.

Each reaction contained 0.75 µl $MgCl_2$ mM, 0.5 µl (10 mM) dNTPs, 1.5 µl of each primer (10 pmol/µl), dH_2O 13 µl, approximately 5 µl template DNA 50 U/µl *Taq* DNA polymerase 0.25 µl and 10× buffer in a total volume of 25 µl PCR products were purified using centrifugal devices (Spectrafuge 16M) and sequenced.

A BLAST search of the NCBI Genbank Database was then performed to identify species or strains of closest similarity. An initial BLAST search of the NCBI Genbank Database against the sequence data described above provided candidate sequences from which we compared the cyanobacteria of this study to previously characterized species. Multiple alignments were then created with reference to the selected Genbank sequences using BioEdit version 7.0.031 which implements the ClustalW multiple alignment algorithm (Higgins *et al*. 1994). Alignment positions at which one or more sequences had gaps or ambiguities were omitted from the analysis.

Results

- Cyanobacterial morphotypes

In this study, 15 distinct morphotypic species of cyanobacteria were characterized using light microscope (Fig. 1). The mat samples from Nazloochia showed the most diverse morphotype assemblages, followed by those of Zarineh River, Rahmanloo Port, Talkheh River and Golmankhaneh Port. The most abundant forms in Urmia Lake were *Oscillatoria* sp., *Phormidium* sp., *Nostoc* sp. and *Microcoleus* sp..

Three species of cyanobacteria isolated from Rahmanloo Port: *Spirulina maxima*, *Gloeocapsa* sp. and *Nostoc* sp., one from Golmankhaneh Port, *Oscillatoria* sp. and two strains from Zarineh River, *Nodularia spumigena* and *Nostoc* sp.. More species were isolated from Nazloochia: *Phormidium fragile*, *Oscillatoria salina*, *Plectonema notatum*, *Aphanocapsa grevillei*, *Oscillatoria* sp., *Trichormus variabilis*, *Leptolyngbya* sp., *Chroococcus turgidus*, *Lyngbya* sp., *Microcoleus chthonoplastes*, *Microcoleus* sp. and *Chroococcus dispersus* were isolated from Talkheh River (Tables 2 & 3).

Table 2. Survey of cyanobacteria diversity isolated from stations 1: Rahmanloo Port, 2: Golmankhaneh Port, 3: Zarineh River, 4: Nazloochia ana 5: Talkheh River

Cyanobacteria	Stations				
	1	**2**	**3**	**4**	**5**
Trichormus variabilis	–	–	–	+	–
Aphanocapsa grevillei	–	–	–	+	–
Chroococcus dispersus	–	–	–	–	+
Chroococcus turgidus	–	–	–	+	–
Gloeocapsa sp.	+	–	–	–	–
Leptolyngbya fragilis	–	–	–	+	–
Leptolyngbya notata	–	–	–	–	–
Leptolyngbya sp.	–	–	–	+	–
Lyngbya sp.	–	–	–	+	–
Microcoleus chthonoplastes	–	–	–	–	+
Nodularia spumigena	–	–	+	–	–
Nostoc sp.	+	–	+	–	–
Oscillatoria sp.	–	+	–	+	–
Phormidium salinum	–	–	–	+	–
Spirulina maxima	+	–	–	–	–

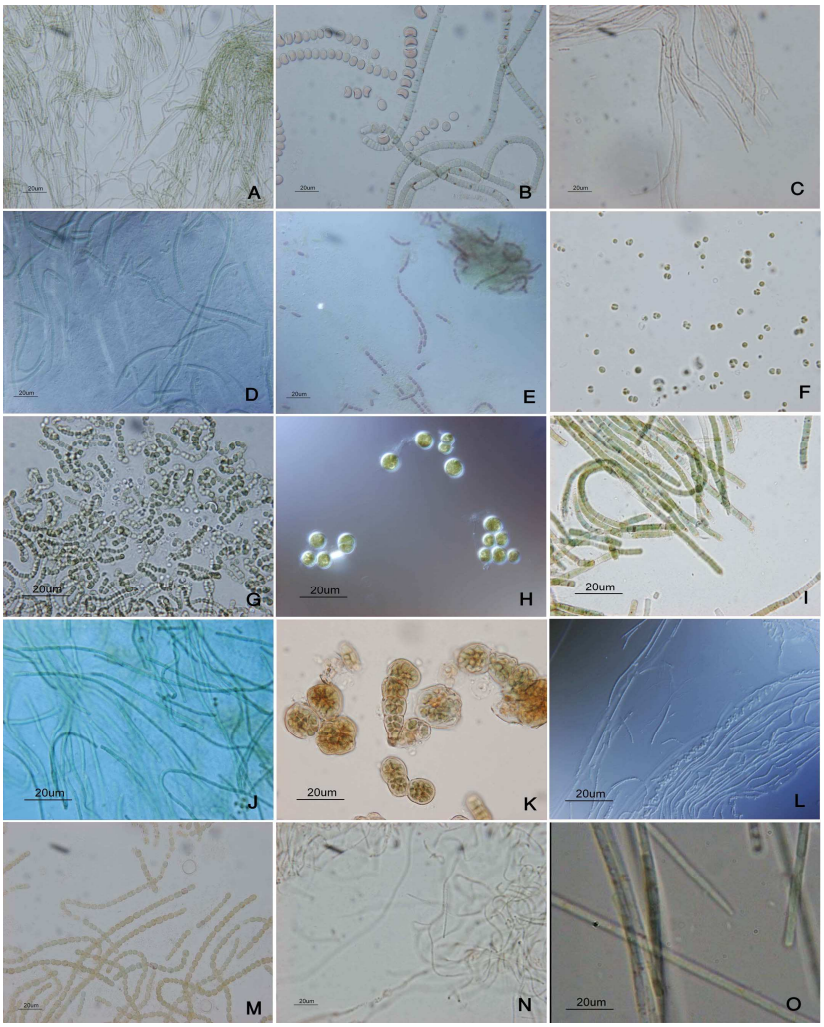

Fig. 1. Cyanobacterial morphotypes from Urmia Lake in Iran: A. *Leptolyngbya* sp., B. *Nodularia spumigena*, C. *Phormidium salinum*, D. *Spirulina maxima*, E. *Microcoleus chthonoplastes*, F. *Chroococcus dispersus*, G. *Nostoc* sp., H. *Chroococcus turgidus*, I. *Oscillatoria* sp., J. *Microcoleus* sp., K. *Gloeocapsa* sp., L. *Phormidium* sp., M. *Trichormus variabilis*, N. *Lyngbya* sp., O. *Leptolyngbya notata*.

Isolated cyanobacteria in Nazloochia cyanobacteria were:

Phormidium fragile (Fig. 1L), trichome mucilaginous, lamellated, yellowish or brownish, blue-green, sheath diffluent; trichome more or less flexuous, entangled or nearly parallel, attenuated at the ends, 1.2–2.3 µm broad. Cells nearly quadrate, 1.2–3 µm long; end-cell acute-conical, calyptra absent.

Leptolyngbya sp. (Fig. 1.A), trichome straight or, if sinuous, for only a portion of length sheath persistent and thick (usually> 1 µm); slow or transient motility; trichome diameter <3 µm.

Microcoleus chthonoplastes (Fig. 1.E), two to several trichomes oriented parallel. Often spirally and tightly interwoven, are encolosed by a common homogeneous sheath. Cells (diameter 3–6 µm) composing, trichomes are longer than wide (or isodiameteric), visible constrictions at the cross-walls, end-cells are conical; the bundles of filaments generally exhibit slow gliding motility, not involving rotation. One genus isolated from saline and hyper saline habitat (up to~100 g/L).

Trichormus variabilis (Fig. 1.M), trichome straight, trichome composed of barrel-shaped or cylindrical cell; motile or immotile; end-cells conical (gliding species) or undifferentiated (planktonic members); heterocysts exclusively intercalary, or both intercalary and terminal; akinetes adjacent to or remote from, heterocysts; gas vesicles in some members.

Oscillatoria salina (Fig. 1.C), trichome straight, elongate, erect, scarcely curved, fragile rapidly moving, 3–5 µm diameter, apices of trichome straight, hooked or twisted, not capitates, apical cell mucronate hyaline, calyptra absent; cells shorter than broad, 1.5–2 µm long.

Chroococcus turgidus (Fig. 1.H), cells spherical or ellipsoidal single, or in groups of mostly 2–4, very seldom many, blue-green, olive green or yellowish, without sheath 8–32 µm, with sheath 13–25 µm diameter, rarely 40 µm; sheath colorless, not distinctly lamellated, planktonic. In mangroves, attached to sub-merged parts, subaerial on tree trunks.

Oscillatoria sp. (Fig. 1.I), trichome straight and divided exclusively by binary fission and in one plane. Cylindrical cells; flexible or semi-rigid; over 4 µm in diameter; disc-like cells wider than long. Reproduction of trichomes is by transcellular breakage. The trichome exhibits gliding motility by rotation; sheaths are nearly invisible. The color is variable, ranging from bright blue-green to deep red. The terminal cells of many species of *Oscillatoria* are differentiated to a shape distinct from the simple bulging of unattended cross-wall. Shapes include round, blunt, conical, prolonged-attenuate, capitate and calyptera.

Lyngbya sp. (Fig. 1.N), trichome straight or, if sinuous, for only a portion of length, trichomes more or less ensheated and, if motile, do not seem to rotate; some members produce short hormogonia that migrate out of the sheath, in others a distinct hormogonial phase cannot be recognized; sheath persistent and thick (usually> 1 µm); single trichome per sheath; trichome width typical, 8 µm; motility restricted to hormogonia.

Plectonema notatum (Fig. 1.O), filaments variously bent, not forming any distinct thallus, 1.7–2 µm broad; false branching sparse, single or geminate; sheath thin, colorless, cells cylindrical.

Aphanocapsa grevillei, tallus gelatinous, spherical or hemispherical, light blue-green; cells spherical, 3.2–5.6 µm diameter, contents finely granular, blue-green, closely arranged in a homogrneous mucilage; individual envelopes not distinct.

Isolated cyanobacteria in Rahmanloo Port were:

Spirulina maxima (Fig. 1.D), trichome helix usually open, cylindrical; cross wall are visible with light microscopy; cell width typically 6–12 µm; gas vesicles generally present.

Gloeocapsa sp. (Fig. 1.K), cells spherical, 3–10 µm in diameter occurring in aggregates; reproduction by transverse binary fission in two planes; structured sheath present.

Nostoc sp. (Fig. 1.G), trichome straight, motile or immotile hormogonia differentiate terminal heterocysts at both extremities; growth leads to wave-like twisted or tightly coiled, immotile mature trichomes with additional intercalary heterocysts; akinetes in chains, initiated distant from heterocysts; transient gas vesicle formation in hormogonia of some members.

In Zarineh River, cyanobacteria isolated were:

Nodularia spumigena (Fig. 1.B), trichome straight, trichome composed of discoid cells, often surrounded by a thin sheath; motile or immotile; heterocysts intercalary and terminal; akinetes in chains, usually initiated distant from heterocysts; gas-vesicles in some members.

Nostoc sp. (Fig. 1.G) and *Chroococcus dispersus* (Fig. 1.F), cells 4–8, 16 or more in a tabular mucilaginous free-swimming colony, with round margins, either solitary and then widely separated from each other or in groups isolated from each other or brilliant blue-green, without sheath 3–4 µm broad, with sheath 5–6 µm diameter, colonies or groups 15–20 µm distant; individual envelopes often totally gelatinized, not lamellated and colourless.

Table 3. Morphological traits of cyanobacterial studied (all traits reported correspond to cultures grown at 30° C)

Cyanobacteria	Cell		Division			Halotolerance category	Salinity range % NaCl	Morphotype	Sample type
	Width (µm)	Length (µm)	Shape	Plane (s)	Asymmetry				
Nodularia spumigena	5	3	Disc-shaped	1	+	Halotolerant	0-25	Heterocystous, Long filaments, acuminate apical cell	Soil
Nostoc sp.	6	5	Elliptical	1	+	Halotolerant	0-10	Heterocystuos, Long filaments	Soil
Gloeocapsa sp.	5	3	Spherical	2	_	Halotolerant	0-45	Unicellular, firm sheath, 4 more	Pool (sediment)
Chroococcus turgidus	5.1	8.4	Coccoid	2	_	Halotolerant	0-20	Unicellular, 2-4	Pool (sediment)
Oscillatoria salina	1.5	2	Bacilloid	1	+	Halotolerant	0-6	Non-heterocystous, Long filaments, barrel-shaped cells	Pool (sediment)
Oscillatoria sp.	7	5	Disc-shaped	1	+	Halotolerant	0-25	Non-heterocystous, Long filaments	Soil
Spirulina maxima	4	3	Disc-shaped	1	+	Halotolerant	0-10	Non-heterocystous, Long, heliacal filaments	Pool (sediment)
Trichormus variabilis	3	5.2	Elliptical	1	+	Nonhalotolerant	0	Heterocystous, Long filaments	Soil
Chroococcus dispersus	2.1	3.1	Coccoid	2	_	Nonhalotolerant	0	Unicellular, 2-4	Pool (sediment)
Leptolyngbya	1	1.8	Bacilloid	1	+	Nonhalotolerant	0	Non-heterocystous, Long filaments, facultative sheath	Soil
Lyngbya sp.	1.2	2	Bacilloid	1	+	Nonhalotolerant	0	Non-heterocystous, Long filaments, facultative sheath	Soil
Phormidium fragile	1.5	2.3	Bacilloid	1	+	Nonhalotolerant	0	Non-heterocystous, Long filaments	Soil
Microcoleus chthonoplastes	2	3	Bacilloid	1	+	Halointolerant	0-6	Non-heterocystous, Long filaments	Soil
Plectonema notatum	1.7	2	Bacilloid	1	+	Nonhalotolerant	0	Non-heterocystous, Long filaments	Pool (sediment)
Aphanocapsa grevillei	3	2	Spherical	2	_	Halotolerant	0-30	Unicellular, colony distinct	Pool (sediment)

Discussion

Most cyanobacteria found in the Urmia Lake have been reported for other saline environments as well. The cyanobacterial species found to dominate in the present study also occur in other saline and hypersaline ecosystems (Javor 1989, Montoya & Golubic 1991, Garcia-Pichel *et al.* 2001, Montoya 2009,). Ryahi *et al.* (1994) observed six cyanophyta (*Anabaena* sp., *Anacystis* sp., *Chroococcus* sp., *Lyngbya* sp., *Oscillatoria* sp. and *Synechococcus* sp.), four chlorophyta (*Ankistrodesmus* sp., *Dunaliella* sp., *Monostroma* sp. and *Pandorina* sp.), two bascillariophyta (*Amphora* sp. and *Navicula* sp.). Mohebbi *et al.* (2006) reported three cyanophyta (*Anabaena* sp., *Oscillatoria* sp. and *Synechococcus* sp.), two Chlorophyta (*Dunaliella* sp. and *Ankistrodesmus* sp.), 11 bascillariophyta (*Navicula* sp., *Nitzschia* sp., *Cyclotella* sp., *Symbella* sp., *Synedra* sp., *Pinnullaria* sp., *Diatoma* sp., *Amphiprora* sp., *Surirrela* sp., *Cymatopleura* sp. and *Gyrosigma* sp.), during monthly samplings over an entire year. These variations may be related to limited and irregular sampling or increased salinity of the lake during recent years that has eliminated some non-tolerant species. Our results are in agreement with some species mentioned above.

The salinity of Urmia Lake has increased dramatically to more than 300 gL^{-1} during recent years and that has greatly influenced almost all aspects of the lake. Population genetic studies which carried out by Mohebbi *et al.* (2006) showed that the south area caused most differentiated populations and the south and median areas of lake have relatively high genotypes (composite haplotypes) distribution in comparison with north area. Accordingly, human population growth in the lake's basin has seriously increased the need for agricultural and potable water in recent years, all of which are supplied from surface and groundwater sources in the area.

Nevertheless, the combination of morphological studies with molecular analysis of natural populations and cultures of cyanobacteria offers a powerful approach to understanding the diversity of cyanobacteria. Their interpretation should allow the description of Micro-organisms as they occur within natural habitats and the identification of phylogenetically coherent taxa.

These variations may be related to limited and irregular sampling, or increased salinity of the lake during recent years that has eliminated some non-tolerant species. Further work is clearly needed to resolve these matters. This study showed that, despite the hyper-salinity and hard condition in Urmia Lake, it is now biologically active.

- Phylogenetic analysis of the cyanobacterial 16S rRNA:

As most micro-organisms miss in culture attempts in the laboratory, we studied the 16S rRNA gene-defined community diversity in cyanobacterial mats from Urmia Lake. All successfully sequenced 16S rRNA gene sequences were blasted against the complete non-redundant NCBI Genbank Database and those sequences found to share a high level of similarity were used to resolve alignment ambiguities and to establish relationships for the sequences obtained in this study. The phylogenetic tree, generated for the samples isolated in this study and the related sequences from the NCBI Database is shown in Fig. 2. The *Scenedesmus* sp. 18S rRNA sequence was used as the outgroup to root the tree.

The overall phylogenetic tree generated from these sequences suggested that the most similar morphotypes may be distinct species with similar morphology.

Since sequences from this study are unlike those recovered from other previously studied regions of the Earth, it would be important to study the human impact on these sites and possibly enact measures to preserve this natural resource. As our results show, there is still quite a bit of room to improve the NCBI taxonomy within the cyanobacteria, but at present these are the currently accepted taxonomic relationships. As additional studies are published, the taxonomy at NCBI is updated to reflect an improved understanding of the phylogenetic relationships of the species in question. Our results clearly show the problem with basing taxonomic relationships on morphological versus molecular based

methodologies. Since we were mainly focused on establishing the overall level of diversity versus the absolute taxonomic relationships, our results clearly establish that there is significant diversity in Urmia Lake and some of this cyanobacterial diversity has not yet been characterized outside of our work. In conclusion, we have characterized community molecular diversity of cyanobacterial mats from five sites in Urmia Lake. It was found that the 16S rRNA gene-defined diversity of all mats exceeded that observed by microscopy alone, showing that the molecular data complements established morphological methods and reveals an increased cyanobacterial diversity over that seen using microscopy alone. From this increased molecular diversity, several morphologically similar yet molecularly distinct cyanobacterial clusters were characterized which displayed both temperature and phylogeographic clines.

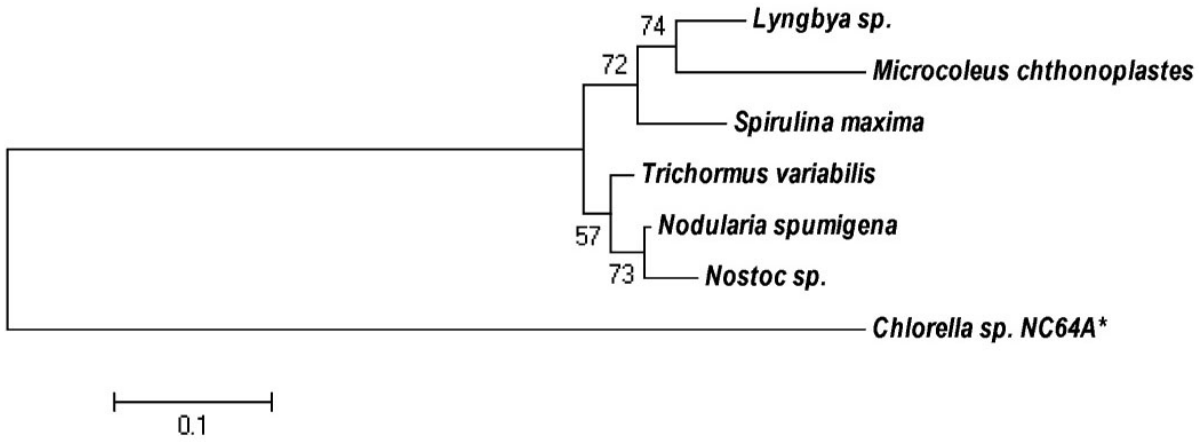

Fig. 2. Phylogenetic tree of the partial 16S rRNA of cyanobacteria of this research (*Chlorella* sp. NC64A* is outgroup).

The 16S rRNAs vary in their nucleotide sequences, but they contain regions that are conserved perfectly or nearly so, among all organisms so far inspected. Certain of these conserved sequences, adjacent to less-conserved regions that are useful for phylogenetic evaluations, provide broadly applicable initiation sites for primer elongation sequencing techniques (Lane *et al.* 1985).

However, the sequence data available for these genes are rather limited, whereas the determination of 16S rRNA gene sequences is a routine procedure in prokaryotic taxonomy today, resulting in large and steadily growing databases which improve the robustness of phylogeny reconstructions, identification results and primer specificity evaluations. Other molecular biological approaches which have been described for the identification of cyanobacteria are applicable exclusively to axenic cultures.

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