

New simple trichal cyanobacterial taxa isolated from radioactive thermal springs

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Abstract: There are several places in the world where the level of natural radiation is unusually high, such as some regions of Ramsar and Abegarm–e–Mahallat in Iran. Such places are still insufficiently explored in terms of their biodiversity. In this study, strains isolated from six geothermal springs with elevated natural radiation were investigated. The highest concentration of ²²⁶Ra were 13,000 Bq.kg⁻¹ in the soil and 130 Bq.l⁻¹ in water respectively. The morphological and molecular analyses revealed, that the strains are not classifiable to any existing taxon. Therefore, they are described as new taxa for science. Based on results of our study, two earlier described species are transferred into newly established genus.

Key words: Cyanobacteria, High–level natural radiation areas, thermal springs, ²²⁶Ra, Ramsar, Iran

INTRODUCTION

Major components of natural ionizing radiation sources are cosmic radiation sources and terrestrial radiation sources. Radiation exposure from terrestrial sources includes external and internal irradiation. Internal irradiation because of inhalation of radium (²²⁶Ra) progenies (e.g., radon and thoron) and ingestion of ²²⁸U and ²³²Th decay series is the main dose participant compared to the external irradiation of terrestrial gamma radiation (TGR). The irradiation of TGR is mainly due to various γ radiation emitters from primordial radionuclides of ²²⁶Ra, ²³²Th series and ⁴⁰K in soil. In fact, diffusion of radionuclide concentrations is associated with local geology and geographical factors of places (SANUCI et al. 2016).

Organisms inhabiting the world's High Background Natural Radiation Areas (HBNRAs) and radon prone areas are exposed to radiation doses that are relatively higher than doses in normal background radiation areas (NBRAs) (SOHRABI 2013b). Some of the world's HBNRAs are located in Kerala (India), Guarapari (Brazil), Yangjiang (China) and Ramsar and Mahallat (Iran) (MORTAZAVI et al. 2002; MORTAZAVI & MOZDARANI 2012).

The world's HBNRAs have been classified on the basis of inhabitants' annual effective dose (HE): low (HE < 5 mSv.y⁻¹), medium (HE= 5–10 mSv.y⁻¹), high

(HE = 20–50 mSv.y⁻¹) and very high (HE > 50 mSv.y⁻¹) (SOHRABI 1998). This grouping is based on the dose limits of the International Commission on Radiological protection (hereafter ICRP) and the 2.4 mSv.y⁻¹ global mean dose value reported by UNSCEAR (ICRP 1991, 2007; UNSCEAR 2000a, b). Specific characteristics of HBNRAs depend on the stability of natural radioactivity. There are areas in which the radiation dose is constant over time and other areas in which the dose varies with time (SOHRABI 2013a).

Ramsar, the city in northern Iran, is a high–level natural radiation area and Mahallat, the city in central part of Iran, is a medium–level natural radiation area. The elevated level of natural radioactivity in Ramsar and Mahallat is caused by ²²⁶Ra and its decay products, which have been brought up to the earth's surface by water of thermal springs. There are nine and five geothermal springs with varying concentration of radium (²²⁶Ra) in Ramsar and Mahallat respectively, which are used as spas (SOHRABI 1993, 1994; GHIASSI–NEJAD et al. 2002; ALIYU & RAMLI 2015).

Geothermal springs are great examples of extreme habitats for organisms with 'subcosmopolitan' geographical distribution (species occurring throughout the world but only in 'appropriate' habitats) (FINSINGER et al. 2008). The hot spring mat communities in terrestrial habitats, as life in extreme environments, have long enticed the consideration of microbial

ecologists because of unique adaptations, diversity of organisms and molecular and regulatory mechanisms to these harsh environments (ZAKARIA et al. 2008; GLAZIER 2009).

Products acquirable from extremophiles, such as proteins, enzymes and other compounds, are of significant interest to biotechnology. This field of research has also attracted attention due to its impact on the possible existence of life on other planets. (SATYANARAYANA et al. 2005).

Microorganisms have been isolated from extreme environments ranging from the drought conditions in the Atacama Desert (JAAKKOLA et al. 2016), sea-ice brine and high salinity (LIU et al. 2016), and hot springs (GEESEY et al. 2016). These microorganisms have unique strategies for survival (SHIVLATA et al. 2015).

Cyanobacteria show vast diversity in terms of their morphology, habitat and functionalities (BECH et al. 2012), which are reflected in their genome and/or other related genomic features (e.g., genome size, coding region, GC content). PRABHA et al. (2016) investigated cyanobacterial genomes and their role in environmental adaptations. They showed that habitat is one of the major factors in giving the shape to the functional composition of cyanobacterial genomes towards their ecological adaptations. The cell envelopes in cyanobacteria play a critical role in their adaptation to higher temperatures, especially the cell structure and function (RAHMAN et al. 2008). Among phototrophic microorganisms, Cyanobacteria are an important source of various products of commercial, toxicological and pharmaceutical interest. They are believed to be one of the richest sources of novel bioactive compounds (MAHAJAN et al. 2016).

Cyanobacterial composition of microbial mats from other hot springs have been studied extensively (e.g. CASTENHOLZ 1996; WARD et al. 1998, 2012; SOMPONG et al. 2005; JING HONGMEI et al. 2005; ROESELERS et al. 2007; MCGREGOR & PAUL RASMUSSEN 2007; FINSINGER et al. 2008; OREN et al. 2009; SIANGBOOD et al. 2011; DADHEECH et al. 2013; GHOZZI et al. 2013; BRAVAKOS et al. 2016), but data from radioactive thermal springs are rather scarce. Some data from such habitat are provided e.g. by ANITORI et al. (2000)

Cyanobacterial community living in geothermal springs in the HBNRAs in Iran are special extremophilic microorganisms. They are involved in a landscape-forming process that creates unique environments with a variety of microenvironments, which, in turn, support diverse specialized and poly-extremophilic (thermophilic/radioresistant) organisms. Despite this, biodiversity of cyanobacteria in geothermal springs in HBNRA in Iran has not been studied up to now. This study aimed to isolate, cultivate and characterize novel cyanobacterial strains that colonize hot springs in HBNRAs in Iran. The taxonomic position of

the isolates was evaluated by morphological and molecular phylogenetic analyses. In this study, not only an attempt has been made to investigate the radioresistant cyanobacterial communities, but also new cyanobacterial taxa that occur in extreme environments of thermal springs in HBNRAs in Iran have been introduced, which will be invaluable for their adaptations and potential biotechnological applications.

To the best of our knowledge, this is the first report on the cyanobacterial diversity isolated from HBNRAs in Iran.

MATERIAL AND METHODS

Site description and sample collection. In this study, water and soil samples were collected from 10 thermal springs near Ramsar (Mazandaran Province, Iran) and Mahallat (Markazi Province, Iran), which are known as HBNRAs (areas known to have the highest ^{226}Ra and gamma exposure levels in Iran). Geographically, Ramsar is located at $36^{\circ}53'N$ $50^{\circ}41'E$, with an average altitude of 20 m a.s.l. (SOHRABI 2013b). The other studied area, Mahallat, is located at $34^{\circ}00'N$ $50^{\circ}33'E$, with an average altitude of 1860 m a.s.l. (BEITOLLAHI et al. 2007). Water samples were collected via the method of DADHEECH et al. (2013) for culturing cyanobacteria. For the purpose of the chemical analysis, the water samples were stored in polyethylene containers previously conditioned with 0.03 mol.l^{-1} HNO_3 and stored at 4°C until the usage. Soil samples were collected from the hot springs at a depth of 5 cm to determine their natural radionuclide content. The collected soil samples were sieved and dried.

^{226}Ra determination and chemical analysis. Determination of the ^{226}Ra activity in the water samples was carried out via radon emanations, according to the procedure described by FORTE et al. (2004). ^{226}Ra was measured by measuring its daughter ^{222}Rn after reaching equilibrium with its parent (about 30 days). The method is based on ^{222}Rn extraction via a scintillation cocktail, immiscible in water, followed by measurement of the alpha and beta emissions, with a liquid scintillation counter, of the ^{222}Rn and its decay products in radioactive equilibrium with the parent.

The collected soil samples were held in sealed Marinelli beakers for a minimum of three weeks (the time required to achieve secular equilibrium between ^{226}Ra and its daughter products). The ^{226}Ra activity of the soil samples was consummated by direct gamma spectrometry (at 186 keV energy) using a high-purity Ge-detector and a multi-channel analyzer. The counting time for all samples was set to 60,000 sec. Local spring water temperature was measured in situ using a laboratory thermometer (Mercury thermometer). Atomic absorption spectrophotometer (AAS, Varian AA-220) was applied to determine metals such as Ca^{2+} , Mg^{2+} , Mn^{2+} , Ni^{3+} , Na^{+} and K^{+} in the water samples. The pH of the water samples was determined by a digital pH meter (DENVER pH meter), with the samples' electrical conductivity determined using a conductivity bridge. Measured values are listed in Table S1.

Isolation and Morphological evaluation of cyanobacteria. Water and soil samples were collected from 10 thermal springs across the areas selected for the study. The water

samples were streaked on agar with BG–11 medium (Stanier et al. 1971). The Petri dishes were incubated in a culture chamber with the following parameters: 25 °C by application of 12/12 h light/dark cycles by artificial illumination (30 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for two weeks. After colonization, resulting isolates were transferred to new agar plates for purification. Isolated cyanobacterial strains were identified using the KOMÁREK and ANAGNOSTIDIS (1999, 2005), and KOMÁREK (2014). Taxonomic determination was carried out by light microscopy on Olympus BX 53.

DNA processing. The genomic DNA was isolated in accordance with the modified xanthogenate–SDS buffer extraction protocol with addition of 3% PVPP and PEG–MgCl₂ precipitation (Yilmaz et al. 2009). A segment of partial SSU rRNA gene and the associated 16S–23S internal transcribed spacer (ITS) region was amplified using primer 1 (5′–CTC TGT GTG CCT AGG TAT CC–3′) (WILLMOTTE et al. 1993) and primer 16S27F (5′–AGA GTT TGA TCC TGG CTC AG–3′) (Lane et al. 1991). Each PCR reaction consisted of 12 μl of Plain Combi PP Master Mix (Top–Bio, Czech Republic), 1 μl of each primer ($c = 5 \text{ nm}$), 20–30 ng of template DNA and 12 μl of sterile bidistilled water. PCR protocol was as follows: 5 min of initial denaturation (94 °C), followed by 40 cycles of denaturation (1 min at 94 °C), annealing (45 s at 52 °C) and elongation (2 min at 72 °C), and final extension for 10 minutes at 72 °C. PCR products were cloned into *E. coli* using the pGEM–T Easy (Promega, Madison, WI, USA) vector system, and the resulting plasmids were purified using NucleoSpin Plasmid kit (Macherey–Nagel, Düren, Germany), and sequenced in SeqMe, s.r.o. (Dobříš, Czech Republic) using standard plasmid primers (T7promoter and SP6r).

Phylogenetic analyses. In order to reveal phylogenetic relations of strains of interest to other simple trichal Cyanobacteria from orders Synechococcales and Oscillatoriales (sensu KOMÁREK et al. 2014), the obtained sequences of the 16S rRNA gene were analyzed together with other sequences available in main repositories (GenBank, EMBL, ENA, DDBJ). Datasets have been built from the most similar sequences found using Blast Search and complemented with sequences of representatives of relevant simple trichal groups. The sequences were aligned using an on–line version of MAFFT v. 7 (<http://mafft.cbrc.jp>; KATO & TOH 2010) with default settings. Minor changes were done manually using BioEdit 7.1.9 (HALL 1999). A fragment of 1160 nt was used for the phylogenetic analysis. In total, this alignment contained 284 sequences (all > 1000 bp in length) including *Gloeobacter violaceus* PCC 7421, PCC8105 and VP3–01 as an outgroup. The pairwise sequence differences were calculated with Geneious 9.1.5 (Biomatters, Auckland, New Zealand). The phylogenetic analyses included maximum likelihood analysis using web IQTree (<http://iqtree.cibiv.univie.ac.at/>; TRIFINOPOULOS et al. 2016), parsimony using MEGA 7 (KUMAR et al. 2016) and Bayesian Inference in MrBayes 3.2.4 (RONQUIST et al. 2012). In the Bayesian analysis, two runs of eight Markov chains were executed for four million generations with default parameters, sampling every 100 generations (the final average standard deviation of split frequencies was lower than 0.01) and first 25% of sampled trees were discarded as burn–in. The maximum likelihood analysis was conducted applying general time reversible model + invariant + gamma (GTR+I+ Γ) chosen according to Akaike Information Criterion values given by jModelTest2

(DARRIBA et al. 2012). The relative support of branches was determined by 1000 bootstrap pseudoreplicates. The secondary structures of D1–D1' and box B helices of the ITS region were predicted using Sfold application server (DING et al. 2004) version 2.2. Secondary structures folded for all newly described taxa are depicted in Figure 2.

RESULTS AND DISCUSSION

Morphological evaluation of natural samples

According to the morphological examination, *Phormidium* spp. and *Leptolyngbya* spp. together with *Thermoleptolyngbya laminosa* were found to be dominant genera in the hot springs and soil samples. The dominant morphotypes in benthos of the most radioactive spring in Ramsar were *Leptolyngbya* spp. and *Nostoc* spp., in addition the soil with highest radioactivity of 13000 Bq.kg^{–1} was settled by *Cylindrospermum licheniforme*. Morphological and molecular analyses revealed several new taxa to science. Comparison of our results with those reported by other authors is rather difficult, since we are not aware of any paper on cyanobacterial diversity in similar habitat. Some data provides the work by ANITORI et al. (2002) from Paralana hot spring in Australia, but only superficial. The spring also differs from those studied in this paper – in the source of radioactivity (Rn instead of Ra) and has higher temperature.

New cyanobacterial taxa from thermal springs with high radioactivity

Plankthothrix iranica Heidari et Hauer sp. nov. (Fig. 3A, B)

Description: Thallus macroscopic brown–green mats. Filaments solitary, isopolar, straight, dark blue–green or brown–green, without sheath. Trichomes cylindrical, motile, not constricted or very slightly constricted at the not granulated cross–walls, not attenuated to the ends. Cells 4–6 μm in width, shorter than wide or sometimes isodiametric, cell content blue–green, without aerotopes, and apical cells rounded, not tapered, nor capitate, without calyptra, with granular content mainly in older cells. Division by necridic cells and formation hormogonia.

Holotype here designated: CBFS A–82–1, an exsiccate of strain AS S8A

Habitat: Benthic mat in the thermal spring (28 °C) with high natural radioactivity, Abe siah, Ramsar, Iran.

Etiymology: the species is named after the country of its origin.

The strain is morphologically and by its benthic life form most similar to *Plankthothrix clathrata* (Skuja) Anagnostidis et Komárek, but differs in motility and ecology, since *Pl. clathrata* has been described in lakes in Sweden. From other members of the genus this new species (*Pl. iranica*) differs in morphology – absence

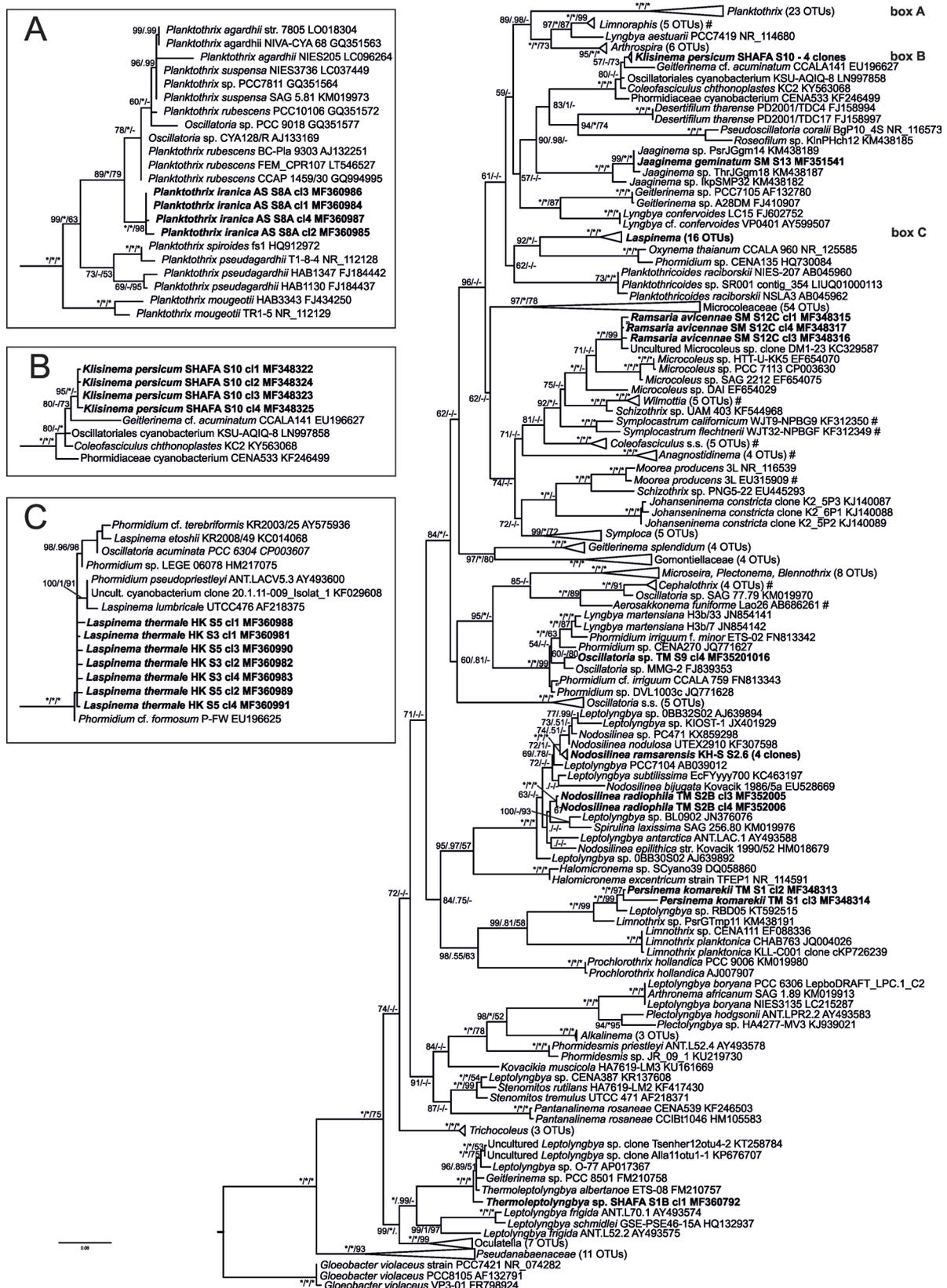


Fig. 1. Phylogenetic analysis of 286 sequences of 16S rRNA gene from 286 strains of simple trichal Cyanobacteria showing position of newly described taxa (typed in bold). The tree is based on maximum likelihood topology and the support values are given for maximum likelihood, Bayesian posterior probabilities, and maximum parsimony. The support values lower than 50, or 0.5 respectively are not shown.

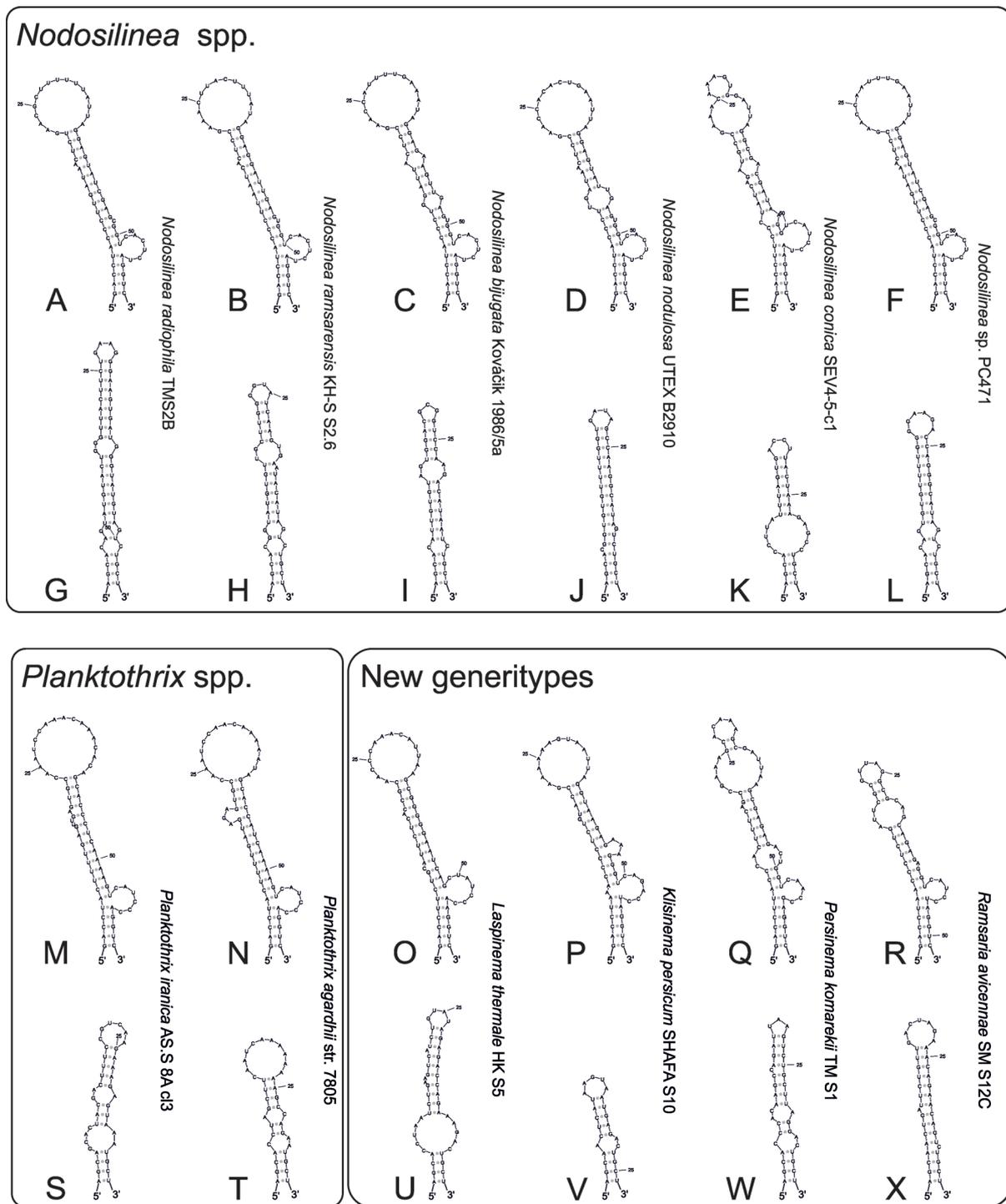


Fig. 2. Comparison of predicted secondary structures of D1–D1' (first structure in pair) and box B helices (second structure in pair) in the ITS regions for *Nodosilinea* spp. and *Planktothrix* spp., and corresponding structures for newly described generitypes: (A, G) *N. radiophila* sp. nov., strain TM S2B; (B, H) *N. ramsarensis* sp. nov., strain KH-S S2.6; (C, I) *N. bijugata*, strain Kováčik 1986/5a; (D, J) *N. nodulosa*, strain UTEX B2910; (E, K) *N. conica*, strain SEV4-5-c1; (F, L) *N.* sp., strain PC471; (M, S) *P. iranica* sp. nov., strain AS.S 8A; (N, T) *P. agardhii*, strain 7805; (O, U) *Laspinema thermale* sp. nov., strain HK S5; (P, V) *Klisinema persicum* sp. nov., strain SHAF A S10; (Q, W) *Persinema komarekii* sp. nov., strain TM S1; (R, X) *Ramsaria avicennae* sp. nov., strain SM S12C.

of aerotopes, cells size, end of trichomes and motility; ecology – *Pl. iranica* forms benthic mats in thermal springs and other species of the genus are mostly freshwater and planktic; and in a 16S rRNA gene sequences similarity < 98.3%, which is under threshold

for prokaryotic species according to YARZA et al. 2014. Unfortunately, we are not able to provide comparison of secondary structures of D1–D1' and box B helices of the ITS region with other *Planktothrix* species but one (Fig. 2) because of unavailability of ITS sequences

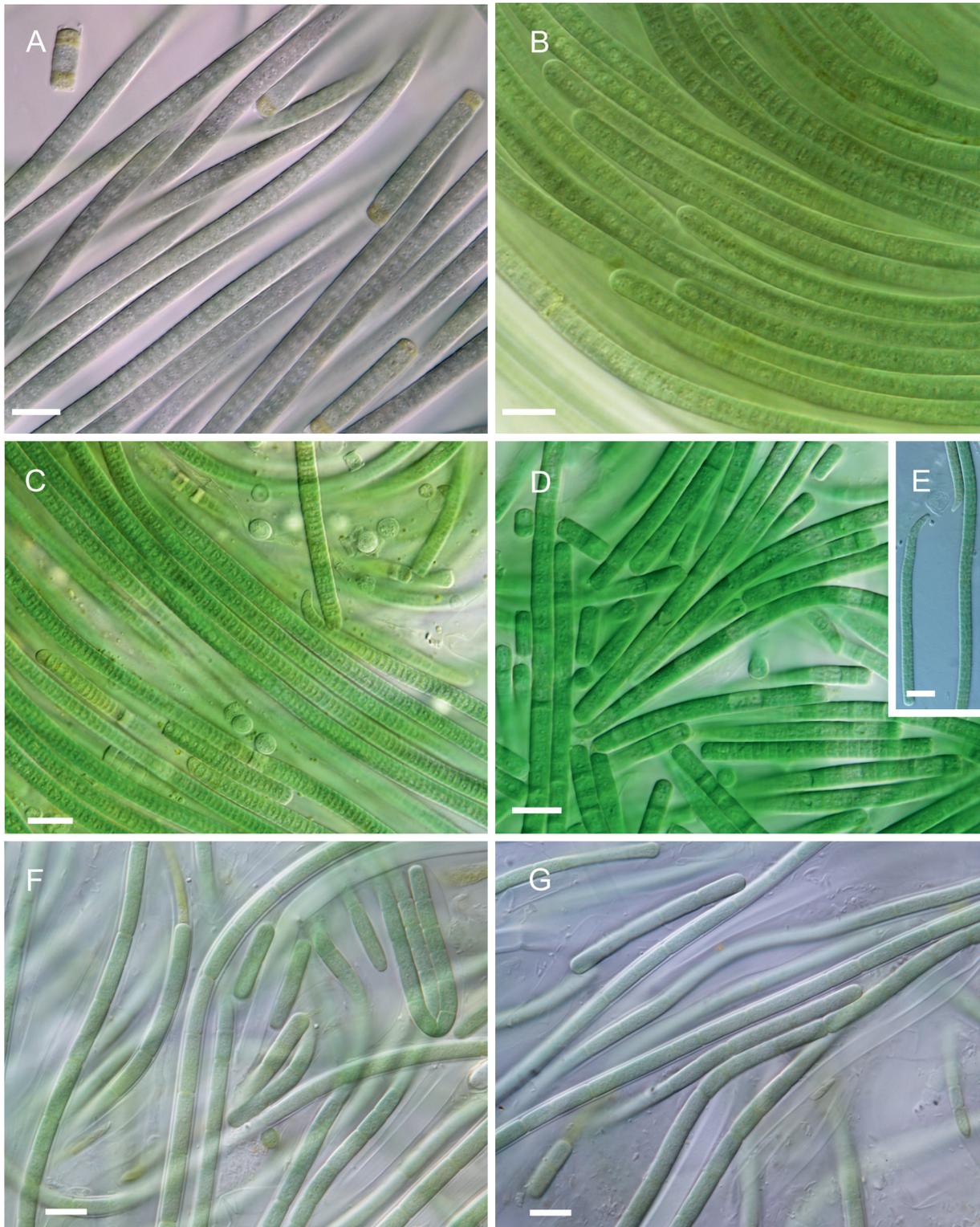


Fig. 3. (A, B) *Planktothrix iranica* sp. nov., strain AS S8A; (C–E) *Laspinema thermale* sp. nov., strain HKS5; (F, G) *Ramsaria avicennae* sp. nov., strain S.M. S12C.

of these taxa. Based on sequence similarities, *Pl. mougeoi* sensu Suda et Watanabe 2002, *Pl. pseudagardhii* Suda et Watanabe, and *Pl. spiroides* Wang et Li could be reclassified outside the genus *Planktothrix*. However, such treatment needs more supporting data and is beyond the scope of this paper.

***Nodosilinea radiophila* Heidari et Hauer sp. nov. (Fig. 5C, D)**

Description: Solitary filaments, straight or wavy, with very thin, colorless sheath. Immotile. Trichomes strongly constricted at the cross walls, 1–2 μm wide. Cells isodiametric or longer than wide, 2–5 μm long,

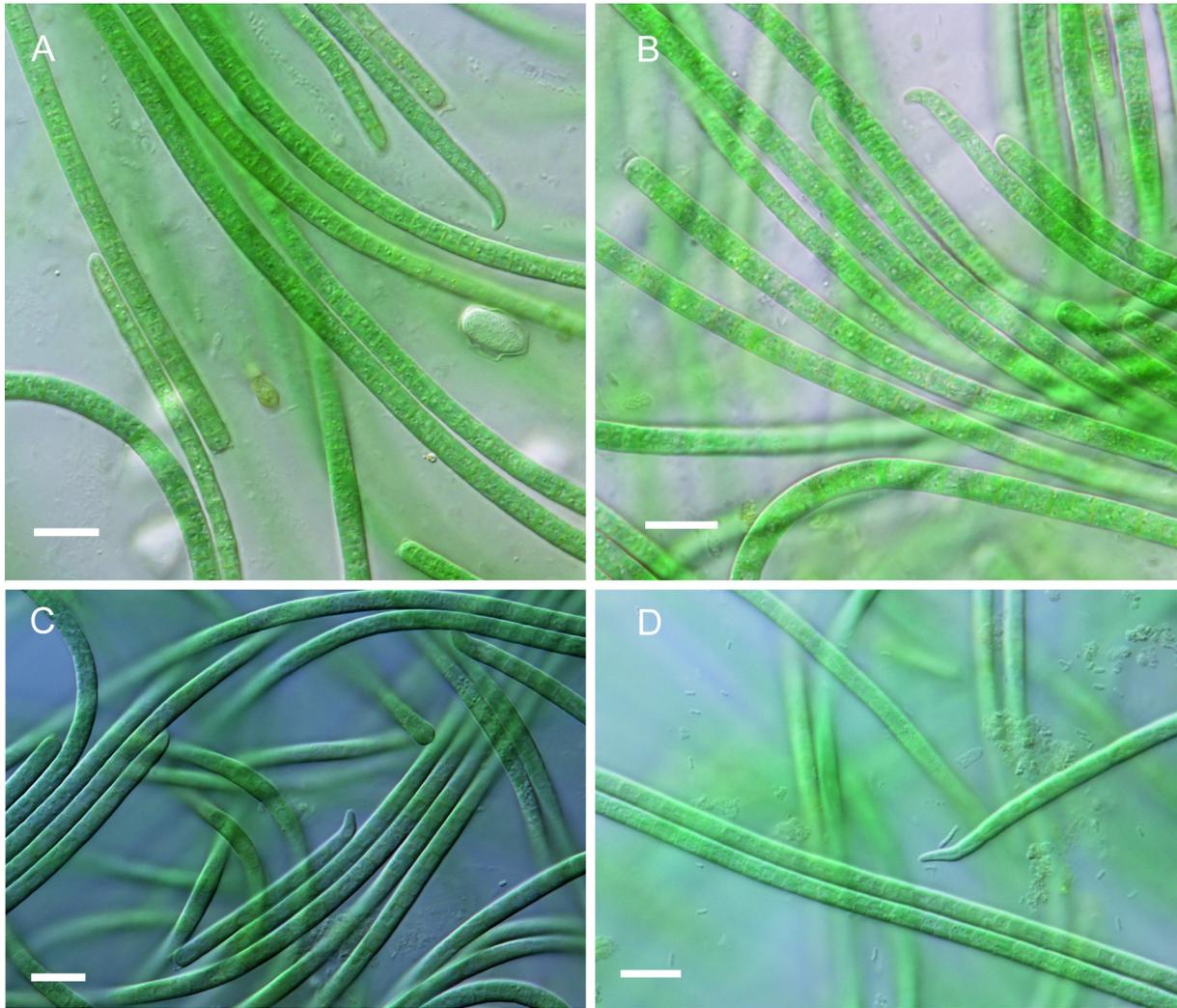


Fig. 4. (A, B) *Klisinema persicum* sp. nov., strain SHAF A S10; (C, D) *Klisinema* sp. strain CCALA 141.

with distinct granules at the cross walls. Reproduction with hormogonia.

Holotype here designated: CBFS A–83–1, an exsiccate of strain TM S2B

Habitat: Benthic mat in the thermal spring (27 °C) with very high natural radioactivity, Talesh Mahalleh, Ramsar, Iran.

Etymology: the specific name refers to the locality of the source material, which contains the highest radiation level measured in the world (Talesh Mahalleh, Ramsar).

From other species of *Nodosilinea*, this organism differs in a 16S rRNA gene sequences similarity < 98.3% and not formation of multiserial trichomes at any time. This organism has distinct granules at the cross walls.

***Nodosilinea ramsarensis* Heidari et Hauer sp. nov. (Fig. 5F, G)**

Description: Solitary filaments, straight, with very thin, colorless sheath. Immotile. Trichomes strongly

constricted at cross walls, (0.8) 1.0–1.5 µm wide. Cells isodiametric or a little longer than wide, 1.0–2.0 µm long, usually with one prominent granule per cell. Nodules are formed very seldom, of multiserial filaments were not observed. Reproduction with hormogonia.

Holotype here designated: CBFS A–84–1, an exsiccate of strain KH–S S2.6

Habitat: Soil around the thermal spring (32 °C), Khak sefid, Ramsar, Iran.

Etymology: the specific name refers to the town of origin of the source material

N. ramsarensis differs from *N. radiophila* in morphology – *N. ramsarensis* has short cells without granules; ecology – *N. ramsarensis* is collected from soil and *N. radiophila* is collected from the mat in thermal spring. Both currently described species differ from those described earlier also in secondary structure of 16S – 23S ITS region, which has been established as a good marker for species differentiation in case of high similarities of 16S rRNA gene sequences. The secondary structures of D1 – D1' and box B helices of current and

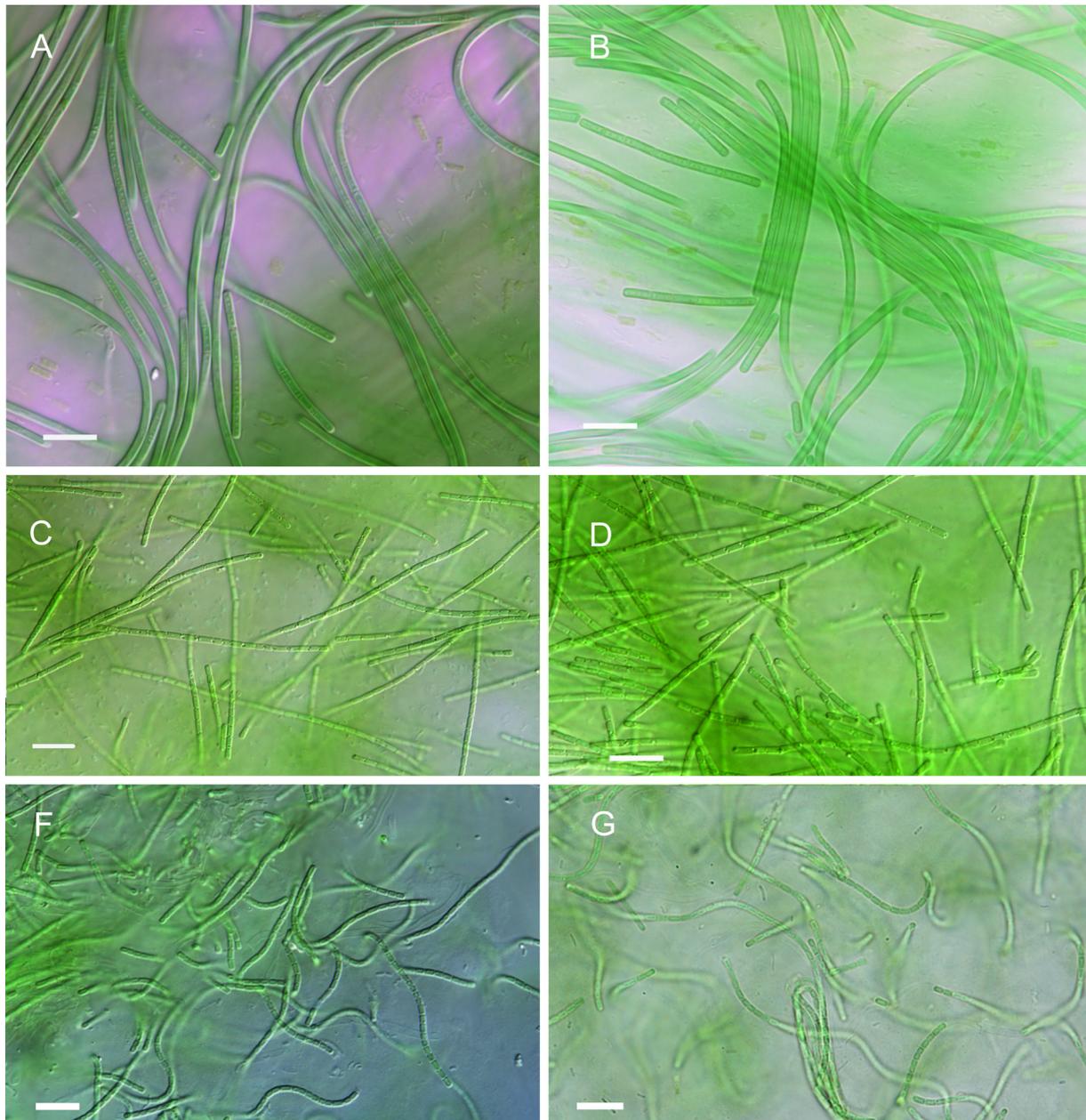


Fig. 5. (A, B) *Persinema komarekii* sp. nov., strain TM S1; (C, D) *Nodosilinea radiophila* sp. nov., strain TM S2B; (E, F) *Nodosilinea ramsarensis* sp. nov., strain KH-S S2.6.

proposed species are in Figure 2.

***Laspinema* Heidari et Hauer gen. nov.**

Description: Filaments straight or wavy, blue–green or olive green. Sheath is thin, inconspicuous. Trichomes cylindrical, isopolar, unbranched, always slightly constricted at cross walls, motile. Cells shorter than wide. The terminal cell is elongated, conical, bent or straight inflated, rounded end without calyptra. Thylakoids have radial arrangement. Aerotopes are not present.

Type species: *Laspinema thermale* Heidari et Hauer sp. nov.

Etymology: Láspi– [Gr.] λάσπη, mud, dirt; –nema (νήμα) [Gr.] – thread; the generic name refers to habitat

of known species and their appearance.

From the most related genus *Oxynema* Chatchawan, Komárek, Strunecký, Šmarda et Peerapornpisal 2012, the proposed genus differs in the level of dissimilarity, which is over 7% in 16S rRNA gene; morphology – *Laspinema* has conical pointed as well as rounded terminal cell contrary to *Oxynema*, which has always sharply pointed terminal cell; motility – *Laspinema* is highly motile contrary to *Oxynema*, which is immotile.

***Laspinema thermale* Heidari et Hauer sp. nov. (Fig 3C, D, E)**

Description: Filaments straight, in natural environment

Table 1. Comparison of main morphological characters and ecology of *Laspinema* species.

	<i>Laspinema thermale</i>	<i>Laspinema etoshii</i>	<i>Laspinema lumbricale</i>
Cell width (µm)	3–4 (5)	5.5 ± 1.5	5–6 (7)
Cell length (µm)	1–2.4	3.0 ± 1.0	2–4
Apical cell	Rounded, conical, shortly bent	Rounded, conical; straight or bent;	Rounded, straight
Habitat	Thermal spring, Iran	Hyposaline water puddles, springs and wet soil, Namibia	Saline pond on McMurdo Ice Shelf, Antarctica
Reference	This paper	DADHEECH et al. 2013	CASAMATTA et al. 2005

forming benthic mats. Trichomes 3–4 (5) µm wide, slightly constricted on cross walls, cells always shorter than wide, terminal cell elongated, conical, bent, never acuminate.

Holotype here designated: CBFS A–85–1, an exsiccate of strain HK S5

Habitat: Benthic mat in thermal springs.

Type locality: Iran, Mahallat (Hakim, 34°00'33"N; 50°33'06"E), material collected by F. Heidari in 2014

***Laspinema etoshii* (Dadheech, Casamatta, Casper et Krienitz) Heidari et Hauer comb. nov.**

Basionym: *Phormidium etoshii* Dadheech, Casamatta, Casper et Krienitz, Fottea 13(2): 237, 2013

***Laspinema lumbricale* (Casamatta, Johansen, Vis et Broadwater) Heidari et Hauer comb. nov.**

Basionym: *Phormidium lumbricale* Casamatta, Johansen, Vis et Broadwater, Journal of Phycology 41(2): 431, 2005

The combinations made above is based on results of phylogenetic analyses and on morphological similarity in main features. Comparison of main characters of all three species is provided in Table 1.

Notes: From *Phormidium* sensu stricto, i.e. the generitype *Phormidium lucidum* Kützing ex Gomont 1892 the newly described genus differs distinctly in morphology of terminal cells – *Laspinema* never has capitulate end cells with calyptra neither thickened cell wall.

***Klisiinema* Heidari et Hauer gen. nov.**

Description: Trichomes straight, isopolar, unbranched, blue–green, motile, with a distinctive oscillation, not constricted or slightly constricted at the ungranulated cross–walls, usually attenuated at the ends. No sheath is present. Cells mostly isodiametric or longer than wide, rarely shorter. Terminal cells strongly bent and acute conical to conical, never pointed. Cell content homogeneous or finely granulated.

Type species: *Klisiinema persicum* Heidari et Hauer sp. nov.

Etymology: Klísi– (κλίση) [Gr.] – bent, –nema (νήμα) [Gr.] – thread; the generic name refers to organism appearance.

From the most similar genus *Oxynema* Chatchawan, Komárek, Strunecký, Šmarda et Peerapornpisal, the proposed genus differs in morphology – *Klisiinema* has strongly bent and acute conical as well as rounded terminal cell contrary to *Oxynema*, which has always sharply pointed terminal cell; motility – *Klisiinema* is highly motile contrary to *Oxynema*, which is immotile. From other similar genus *Laspinema* Heidari et Hauer gen. nov. *Klisiinema* differs in morphology, ecology and genetic features. *Klisiinema* has isodiametric or longer than wide cells, without sheaths, and its natural habitat is soil. These are opposite to *Laspinema* whose cells are never longer than wide, has a fine sheath, and lives in the form of benthic mats in springs.

***Klisiinema persicum* Heidari et Hauer sp. nov. (Fig. 4A, B)**

Description: Trichomes solitary, 3–5 µm wide, bright blue–green, mostly straight, long, without sheaths, intensely motile, not or slightly constricted. Cells isodiametric or longer than wide 3–5 µm long. Terminal cell conical, elongated.

Holotype here designated: CBFS A–86–1, an exsiccate of strain SHAF A S10

Etymology: the species is named after the country of its origin.

Habitat: Soil around thermal springs (50 °C) with high radiation background.

Type locality: Iran, Mahallat (Shafa, 34°00'41"N; 50°32'46"E), material collected by F. Heidari in 2014

In the phylogenetic analyses, SHAF A S10 is in well supported clade with the CCALA 141 (*Geitlerinema* cf. *acuminatum*) among others (box B in Fig. 1). Both strains share similar morphology and ecology. However, SHAF A S10 and CCALA 141 are morphologically completely different from *Geitlerinema*

acuminatum (Anagnostidis et Komárek) Anagnostidis (bent and spherically capitate at the end, end cell elongated up to 15 µm, cells 1.5–3 µm wide and up to 2–4 (6)× longer than wide). A comparison of strain SHAFAS10 with CCALA 141 has shown, that the latter strain should be also classified into the genus *Klisiinema*. Based on figures of the strain CENA 533 in work by ANDREOTE et al. (2014) and its sequence KF246499, we suppose that also this strain could be classified into the genus, but more information would be necessary for confidence.

***Ramsaria* Heidari et Hauer gen. nov.**

Description: Filaments solitary, cylindrical along the whole length, straight, with facultative sheath. Sheaths around single trichomes thick, firm, colorless, not lamellate. Trichomes cylindrical, isopolar, constricted at cross walls, not attenuated towards ends, unbranched. Cells up to 4× longer than wide, cylindrical, pale blue–green, with scattered larger granules. Terminal cells rounded at the apex, without calyptra. Reproduction by disintegration of trichome into immotile segments, sometimes with participation of necridic cells.

Type species: *Ramsaria avicennae* Heidari et Hauer sp. nov.

Etymology: the genus name refers to the name of city of taxon's origin.

In comparison with the most similar genus *Coleofasciculus* (Thuret ex Gomont) Siegesmund, Johansen et Friedl in SIEGESMUND et al. 2008, the proposed genus *Ramsaria* differs morphologically in having always one trichome per sheath in nature as well as *in vitro* and also ecologically in living in freshwater environment. The level of similarity to sequences of *Coleofasciculus chthonoplastes* is < 93.6% in 16S rRNA gene.

From other similar genus *Wilmottia*, our strain SM S12C is distinguished through longer cells, distinct constrictions on cross–walls, and thick sheath. The level of similarity to the reference sequence of *Wilmottia murrayi* (type species of the genus) is < 93.6% in 16S rRNA gene.

***Ramsaria avicennae* Heidari et Hauer sp. nov. (Fig. 3F, G)**

Description: Filaments entangled or fasciculated, flexuous, enveloped by a facultative, thick, homogeneous and colorless sheath, 4–6 µm wide. Trichomes cylindrical, straight, blue–green, constricted at cross walls, not attenuated, immotile, 3.5–4 µm wide. Cells longer than wide, apical cells rounded, without calyptra 5–14 µm long. Cell content blue–green, homogenous, ungranulated cross wall. Sometimes with large granules in the cells.

Holotype here designated: CBFS A–087–1, an exsiccate of strain SM S12C.

Etymology: the species is named after medieval

Persian scholar Avicenna.

Habitat: Benthic mat in thermal springs (35 °C) with high radiation background.

Type locality: Iran, Ramsar (Saddat Mahalleh, 36°53'42"N; 50°41'30"E), material collected by F. Heidari in 2014.

***Persinema* Heidari et Hauer gen. nov.**

Description: Trichomes solitary, isopolar, straight, slightly bent or flexuous. Cells consisting of cylindrical, elongated cells, slightly constricted at cross walls, not attenuated at the ends, without sheaths, motile. All cells with the same morphology, cylindrical, blue–green, without aerotopes. Apical cells cylindrical rounded flattened at the ends. Without calyptra, not capitate. Reproduction by disintegration of trichomes into motile hormogonia, without necridic cells.

Type species: *Persinema komarekii* Heidari et Hauer sp. nov.

Etymology: Persi– refers to Persia, a Middle Eastern ancient, –nema (νήμα) [Gr.] – thread

From the most similar genus *Limnothrix* Meffert, T.M/S1 is obviously different in molecular, morphological and ecological features. T.M/S1 is without aerotopes, motile and constricted at cross walls, these are opposite to *Limnothrix*. Also T.M/S1 was isolated from a mat in the thermal spring and, on the contrary, *L. planktonica* occurs in the freshwater and is planktic.

Note: As already noted by KOMÁREK and ANAGNOSTIDIS (2005), *Limnothrix* is a heterogeneous genus which includes planktic, colder lakes and with polar aerotopes bearing. As the results show (Fig. 1), sequence types designated as *L. planktonica* make a well supported cluster separate from core Pseudanabaenaceae (cluster collapsed in Fig1 1) containing the sequence of type species of the genus, i.e. *L. redekei* (Van Goor) Meffert. Thus, they should be considered as a different genus. It is necessary to note, that morphotypes/strains CHAB736 and KLL–C001 (located in the sister clade to *Persinema*) designated as *L. planktonica* may not be the true *L. planktonica* because of different distribution, which is outside the temperate zone.

***Persinema komarekii* Heidari et Hauer sp. nov. (Fig. 5A, B)**

Description: Trichomes solitary, straight, pale blue green, 1–2 µm wide, constricted at the cross walls, without sheaths. Cells 2–5 times longer than wide (5–8 µm). Without aerotopes. Apical cells cylindrical, rounded.

Holotype here designated: CBFS A–88–1 – dried material from strain TM S1

Etymology: the species is named in honor of Professor Jiří Komárek, a prominent Czech Phycologist and world's famous cyanobacteriologist.

Habitat: Benthic mat in thermal springs (27 °C) with high radiation background.

Type locality: Iran, Ramsar (Talesh Mahalleh, 36°89'90"N; 50°67'49"E), material collected by F. Heidari in 2014

ACKNOWLEDGEMENTS

The research was supported by grant GA CR 15–11912S, and long-term research development project no. RVO 67985939. Access to computing and storage facilities owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum, provided under the programme “Projects of Large Infrastructure for Research, Development, and Innovations” (LM2010005), is greatly appreciated.

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Supplementary material

the following supplementary material is available for this article:

Table S1. Physio-chemical characteristics of soil and water in sampled thermal springs in Iran.

This material is available as part of the online article (<http://fottea.czechphycology.cz/contents>)