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# Morphological and molecular characterization of *Goleter* sp. (Nostocales, Nostocaceae) isolated from freshwater in Iran

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ABSTRACT
To increase the knowledge on diversity of freshwater cyanobacteria, we have characterized a novel filamentous, benthic cyanobacterium isolated from a pond in Golestan province, Iran. The isolate was studied using a polyphasic approach based on morphological and molecular characteristics, including phylogenetic and structural analysis of the 16S rRNA gene sequence and 16S to 23S internal transcribed spacer (ITS), respectively. The overall morphology of the isolate was similar to Nostocalean member's strains with non-tapering filaments and distributed heterocytes. However, the molecular analyses suggested that it was more closely related to the lithobiont and rock-dwelling *Goleter apudmare* Miscoe & J.R.Johansen, which has tapered filaments and basal heterocytes. The 16S rRNA gene phylogenetic analysis, based on bayesian inference and maximum likelihood methods, placed the isolate close to *G. apudmare*. The D1-D1', Box-B and V3 helixes of the isolate's ITS sequence were different when compared to other *Goleter* ITS sequences. Similar to *G. apudmare* HA4356-MV2 (JN385288.1) and *Goleter* sp. CHAB TP201702 (MT488126.1), tRNA genes were absent from the amplified ITS sequence in the studied isolate. These morphological and molecular characteristics differentiate this isolate from all other *Goleter* taxa described so far, which contributes to extend the knowledge on cyanobacterial diversity and occurrence of benthic cyanobacteria in Iranian freshwaters.

KEY WORDS
Cyanobacteria, Nostocales, freshwater, temperate climate, polyphasic approach.

**RÉSUMÉ**

*Caractérisation morphologique et moléculaire de* Goleter *sp. (Nostocales, Nostocaceae) isolée de l'eau douce en Iran.*

Pour accroître les connaissances sur la diversité des cyanobactéries d'eau douce, nous avons caractérisé une nouvelle cyanobactérie benthique filamenteuse isolée d'un étang de la province du Golestan, en Iran. L'isolat a été étudié en utilisant une approche polyphasique basée sur les caractéristiques morphologiques et moléculaires, comprenant l'analyse phylogénétique et structurelle de la séquence du gène de l'ARNr 16S et de l'espaceur transcrit interne (ITS) 16S à 23S, respectivement. La morphologie globale de l'isolat était similaire à celle des souches membres des Nostocales avec des filaments non effilés et des hétérocytes distribués. Cependant, les analyses moléculaires suggèrent qu'il était plus étroitement lié au lithobionte *Goleter apudmar*e Miscoe & J.R.Johansen vivant dans les roches, qui possède des filaments effilés et des hétérocytes basaux. L'analyse phylogénétique du gène de l'ARNr 16S, basée sur les méthodes d'inférence bayésienne et de vraisemblance maximale, a placé l'isolat à proximité de *G. apudmare*. Les hélices D1-D1', Box-B et V3 de la séquence ITS de l'isolat étaient différentes par rapport aux autres séquences *Goleter* ITS. De la même manière que *G. apudmare* HA4356-MV2 (JN385288.1) et *Goleter* sp. CHAB TP201702 (MT488126.1), les gènes d'ARNt étaient absents de la séquence ITS amplifiée dans l'isolat étudié. Ces caractéristiques morphologiques et moléculaires différencient cet isolat de tous les autres taxons de *Goleter* décrits jusqu'à présent, ce qui contribue à étendre les connaissances sur la diversité cyanobactérienne et la présence de cyanobactéries benthiques dans les eaux douces iraniennes.

**MOTS CLÉS**
Cyanobacteria,
Nostocales,
eau douce,
climat tempéré,
approche polyphasique.

## INTRODUCTION

Cyanobacteria are a group of photosynthetic prokaryotic microorganisms that have long captured the scientists' attention. About 5310 species of cyanobacteria have been hitherto described (Guiry & Guiry 2022) and new species have continually been found, described, and typically named according to established rules. The correct determination of cyanobacteria strains concerns new biotechnological applications as well as ecological studies. There are many situations where it is crucial to recognise close species.

The systematics of the phylum Cyanobacteria is challenging and has undergone several revisions during recent years (Komárek & Anagnostidis 1999; Komárek 2013). Moreover, some morphological characters vary considerably in response to different selective pressures, making species delimitation difficult if only based on morphological criteria.

In addition to morphological and physiological characteristics, molecular methods have been used extensively to identify and differentiate cyanobacterial species. In particular, analysis of the16S rRNA gene sequence and 16S-23S rRNA internal transcribed spacer (ITS) secondary structures have enabled taxonomic resolution of novel species (Genuario *et al.* 2015; Miscoe *et al.* 2016; Rigonato *et al.* 2016; Alvarenga *et al.* 2017; Scotta Hentschke *et al.* 2017; de Alvarenga *et al.* 2018; Saraf *et al.* 2018; Kabirnataj *et al.* 2020; Nowruzi & Shalygin 2021; Nowruzi & Soares 2021).

Here, we describe the isolation and characterization of a novel benthic filamentous cyanobacterium collected from a freshwater pond in Golestan province, Iran. The isolate was described morphologically, analyzed phylogenetically based on the 16S rRNA gene sequence, and also evaluated by analysis of the 16S-23S ITS secondary structure. The combined results contribute to extend the knowledge on cyanobacterial diversity and occurrence of filamentous, lithobiont and rock-dwelling cyanobacteria in Iranian freshwaters.

## MATERIAL AND METHODS

### Isolation and morphological characterization

The cyanobacterial material, from which the isolate was obtained, was collected in June, 2016 from a freshwater system (pond) (36°51'25"N, 54°26'55"E), in Golestan province, Gorgan city, Iran. Biomass was collected from submerged stones, placed into cone-shaped plastic tubes (Falcon® conical tubes) and transported to the laboratory for subsequent isolation and identification (Waterbury 2006).

In the laboratory, small sections of the cyanobacterial material were spread onto 1.2% agar-solidified BG-$11_0$ medium (BG-11 without a nitrogen source) (Rippka *et al.* 1979) and cultivated at 28 ± 2°C under an illumination of *c.* 50-55 µmoL photons $m^{-2}s^{-1}$, and a 14:10 h light:dark cycle. The biomass was constantly analyzed under a microscope and successive streaking onto fresh media was performed until a unicyanobacterial colony was obtained. The isolate was temporarily named 121B and maintained in a 250 mL cotton-stoppered Erlenmeyer flask containing liquid BG-$11_0$ medium at 28 ± 2°C with periodic shaking (twice daily), illumination of *c.* 50-55 µmoL photons $m^{-2}s^{-1}$, and a 14:10 h light:dark cycle. Morphological observations (e.g. colony and filament morphologies, vegetative cell, heterocyte and akinete shape and dimensions), set under the provisions of the

International Code of Nomenclature for algae, fungi and plants (McNeill *et al*. 2012), were made using an Olympus CX31RTS5 (Olympus, Tokyo, Japan) stereoscope equipped with a QImaging GO-3 digital camera (Teledyne QIMAGING, Surrey, British Columbia, Canada) and an Olympus BX43 microscope equipped with manufactured Sc50 digital camera. Cell parameters (i.e., vegetative cell, heterocyte and akinete dimensions) were measured using the DP-SOFT software, and from 50 to 200 measurements were taken for each parameter to describe the trait variability. Identification of the isolate was carried out according to Komárek *et al*. (2014) and Komárek (2016a, b). In addition, studies dealing with the description of *Goleter* species were considered (Komárek & Kováčik 1989; Rajaniemi *et al*. 2005; Turicchia *et al*. 2009; Komárek 2013).

### PCR amplification of the 16S rRNA gene sequence and 16S-23S internal transcribed spacer (ITS)

Genomic DNA was extracted from a 16-day-old log phase culture using the HiPurA™ Bacterial Genomic DNA Purification Kit MB505 (HiMedia Lab, Mumbai, India), following the manufacturer's instructions, except for a variation in incubation time in lysis solutions AL (60 minutes) and C1 (20 minutes).

Amplification of the 16S rRNA gene sequence and 16S-23S ITS were carried out separately on the extracted DNA in volumes of 20 µL PCR reaction comprised of 1 X buffer solution (DyNAzyme PCR buffer, Finnzymes, Espoo, Finland), 0.5 µM forward primer (27F or ITS-F), 0.5 µM reverse primer (23S30Ra or ITS-R) (Appendix 1), 0.5 U of Taq polymerase and 10 ng of template DNA. Thermocycling was performed in an iCycler (Bio-Rad, Foster City, CA, United States) (Appendix 1). PCR products were analyzed on 1% agarose gel electrophoresis (SeaPlaque GTG, Cambrex Corp., East Rutherford, NJ, United States) at 100 V, stained with 0.10 µg $mL^{-1}$ of ethidium bromide (Bio-Rad), and visualized under ultraviolet light using a Molecular Imager Gel Doc XR system (Bio-Rad). A digital gel image was obtained with the image analysis software Quantity One version 4.6.7 (Bio-Rad). The size of PCR products was estimated by comparison with DNA molecular marker (λ/HinfIII + λx/HaeIII, Finnzymes).

Cloning of fresh PCR products was performed using the TOPO Ta cloning system with the vector 2.1-TOPO (Invitrogen, Carlsbad, United States) according to the manufacturer's instructions. The cloned inserts were bidirectionally sequenced by Sanger sequencing using the BigDye® terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, California, United States) and internal primers (Appendix 1). Each set of sequencing data was obtained from at least three independent sequencing reactions (Phred ≥ 20). The sequenced fragments were assembled into contigs using the Bio Edit Sequence Alignment Editor Version 7 (Hall 1999), and only bases of high quality were considered.

The 1996 bp assembled sequence, encompassing the full 16S rRNA gene sequence and 16S-23S internal transcribed spacer (ITS), was deposited in the GenBank (National Center for Biotechnology Information) under the accession number OP422450.

### 16S rRNA gene sequence phylogenetic analyses

The boundaries of the 16S rRNA gene sequence from the isolated cyanobacterium were determined using barrnap 0.8 (Seemann 2013). The 1490 bp 16S rRNA gene sequence was queried against the GenBank database (National Centre for Biotechnology Information) using BLASTN (MegaBLAST) search (Altschul *et al*. 1990). The first search was against the standard nucleotide sequence database. The second search was against the 16S rRNA nucleotide sequence database limited to type material. The 16S rRNA phylogenetic tree was constructed using Nostocalean sequences (Appendix 3) aligned with the 16S rRNA sequence from the isolated cyanobacterium using MAFFT version 7 (Katoh & Standley 2013). *Goleter apudmare* Miscoe & J.R.Johansen (KF417425.1) was used as reference sequence, and *Gloeobacter violaceus* Rippka, J.B.Waterbury & Cohen-Bazire (NR074282.1) as outgroup. A maximum likelihood (ML) phylogenetic tree was inferred from the sequence alignment using IQ-Tree multicore version 2.1.2 (Minh *et al*. 2020), with the best-fit model selected by ModelFinder (Kalyaanamoorthy *et al*. 2017) and 10 000 ultra-fast bootstrap replicates. The generated tree was displayed with iTOL (Letunic & Bork 2021) and formatted with Adobe Illustrator 26.3.1.

Additionally, *p*-distances between the 16S rRNA gene sequences of the isolate *Goleter* sp. and its closest phylogenetic relatives were calculated using MEGA11 (Tamura *et al*. 2021). This distance is the proportion (*p*) of nucleotide sites at which two sequences compared are different. It is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared.

### 16S-23S rRNA ITS region secondary structure analysis

The secondary structure of the 16S-23S rRNA ITS of the isolated cyanobacterium was characterized according to Johansen *et al*. (2011). The D1-D1' helix, D2, D3, BOX-B, BOX-A, D4 and V3 regions of the 16S-23S ITS were compared to structures of *Goleter apudmare* (JN385289.1, JN385288.1, KF417425.1) and *Goleter* sp. (MT488126.1, MT488260.1, MT488270.1) using the M-fold web server version 2.3 (Zuker 2003), assuming ideal conditions of untangled loop fix and a temperature of 37°C. The resulting structures were downloaded and formatted with Adobe Illustrator 26.3.1.

### Repositories

Sequence obtained in this study is deposited in GenBank (National Center for Biotechnology Information), under the accession number OP422450.

### Abbreviations

| | |
|---|---|
| ITS | internal transcribed spacer; |
| PCR | polymerase chain reaction; |
| 16S rRNA | small subunit ribosomal RNA. |

TABLE 1. — Morphological comparison of the isolate *Goleter* sp. and its close phylogenetic relative *Goleter apudmare* Miscoe & J.R.Johansen. *Goleter apudmare* characteristics obtained from Miscoe *et al*. (2016).

| Characteristic | *Goleter* sp. (OP422450) | *Goleter apudmare* (KF417425) |
|---|---|---|
| Colony morphology | Densely gelatinous filaments, light green to deep green | Diffuse, light colored, spreading colony, dull grayish blue-green |
| Filament morphology | Flexuous, bent, slightly coiled | Tapering |
| Filament structure | Solitary, polar, non-tapering | Heteropolar, tapering, strongly constricted at crosswalls |
| Branching | Branched | Branched |
| Sheath | Present on some filaments, colorless | Thin, barely visible, colorless |
| Cross-walls | Present | Present |
| Vegetative cell shape | Cylindrical to barrel shaped | Isometric, longer than wide near trichome apex |
| Vegetative cell dimensions (µm) | 5.4 × 4.2-10.1 | 3.8-4 × 2.5-5.5 |
| Vegetative cell granulation | Mostly granular | Homogenous to sparsely granular |
| Heterocyte shape | Ellipsoidal to cylindrical | Solitary, basal, hemispherical |
| Heterocyte dimensions (µm) | 4-4.5 × 5.1 | 2.5-4.4 × 3.6-4.4 |
| Akinete morphology | Spherical, very rare, solitary, in the middle of filaments or free from filaments | Forming in series in basal portion of trichome, homogenous to coarsely granular |
| Akinete dimensions (µm) | 5.1-7.6 × 6.5-7.1 | 3.3-7.5 |
| Akinete granulation | Homogenous to coarsely granular | Homogenous to coarsely granular |
| Hormogonia | Present | Not present |
| Necridia | Occasionally found | Not present |
| Habitat | Lithophytic on stones in freshwater pond, northern Iran | Lithophytic on cave wall, Kauai, Hawaii |

TABLE 2. — Size (bp) of ITS secondary structures of the isolate *Goleter* sp. and related species.

| | D1-D1' helix | Spacer + D2 + spacer | D3 | tRNAIle gene | tRNAAla gene | Box-B | Post Box-B spacer | Box-A | D4 | V3 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Goleter* sp. (OP422450) | 65 | 35 | 4 | – | – | 27 | 17 | 11 | 8 | 21 |
| *Goleter apudmare* HA4356-MV2 (JN385288.1) | 54 | 51 | 3 | – | – | 26 | 17 | 11 | 9 | – |
| *Goleter apudmare* HA4356-MV2 (JN385289.1) | 65 | 38 | 4 | 74 | 73 | 26 | 18 | 11 | 9 | – |
| *Goleter apudmare* HA4340-LM2 (KF417425.1) | 54 | 44 | 4 | 74 | 73 | 26 | 18 | 11 | 8 | – |
| *Goleter* sp. CHAB TP201702 (MT488126.1) | 53 | – | – | – | – | 26 | 16 | 11 | 9 | 29 |
| *Goleter* sp. CHAB TP201702 (MT488260.1) | 53 | 49 | 4 | 74 | 73 | 26 | 16 | 11 | 9 | 29 |
| *Goleter* sp. CHAB TP201823 (MT488270.1) | 53 | 49 | 4 | 74 | 73 | 26 | 16 | 11 | 9 | 29 |

## RESULTS

### MORPHOLOGICAL ANALYSIS

Macroscopically, the isolate grew attached to stones in fresh water, exhibiting pale green to deep green pigmentation (Fig. 1). The culture grew as a gelatinous biomass, forming a wide range of macroscopic colonies on agar plates (Fig. 1D). Further, cell color changed from pale green to deep green with age. In young cultures, unbranched filaments mostly isopolar and untapered were observed, but with at least some trichomes branching. Hormogonia were formed by dissolution of crosswall connections or by formation of necridia, causing fragmentation in the middle of polar trichomes, or fragmentation at polar trichomes, with few-celled hormogonia arising as result. In older cultures, granular cells with vacuolization becoming apparent in senescence were observed. When the cells tear, granules are released. The gross morphology of the isolate was similar to Nostocalean representatives with non-tapering solitary filaments of barrel shaped cells, and dispersed cylindrical heterocytes and akinetes.

However, as the molecular analyses suggested a close relationship between the new isolate and *Goleter* species (see below), a detailed morphological comparison (Table 1) was performed against the type species *Goleter apudmare* (Miscoe *et al*. 2016; Strunecký *et al*. 2023). Surprisingly, the morphology of the isolate differed significantly from that of *G. apudmare* (KF417425). The trichomes of the isolate featured end cells with two corner "spines" and bristles, whereas *G. apudmare* has tapered end cells (Fig. 2A). The cell contents of the isolate were very granular (Fig. 2C), with granules released when


Fig. 1. — **A**-**C**, Nostocaceae cyanobacterium in the original sampled site; **D**, colonies on a Petri dish.

the cells tore. *G. apudmare* cell contents are homogenous to sparsely granular according to Miscoe *et al.* (2016). Necridium cells were occasionally found in the main filaments of the isolate (Fig. 2A, E, G) whereas *G. apudmare* lacks necridium cells. Reproduction in the isolate was observed to occur via uniseriate hormogonia, whereas in *G. apudmare* reproduction occurs via fragmentation of cells. The trichomes of the isolate tend to aggregate into multiseriate clumps (Fig. 2G). *Goleter apudmare* trichomes do not aggregate. Akinetes were rarely observed in the isolate, and were solitary, centrally located in, or free from filaments (Fig. 2E). In *G. apudmare*, akinetes occur in series in the basal portion of trichomes. In the isolate, heterocytes were bone shaped and spherical (Fig. 2G), whereas *G. apudmare* heterocytes are hemispherical. Finally, monocyte reproductive cells were present in the isolate (Fig. 2G), but are absent in *G. apudmare*.

### 16S rRNA gene sequence analyses

The BLASTN search of the 16S rRNA gene sequence of the isolate against the standard nucleotide sequence database returned 50 sequences with identities ranging from 96.53 to 97.99%. All of which were from the order Nostocales. The top hits (> 96.97% identity) were sequences annotated as *Goleter*, *Nodularia* and *Trichormus* genera (Appendix 2). The BLASTN search against the 16S rRNA nucleotide sequence database limited to type material returned 50 sequences with identities ranging from 91.24 to 96.62%, 47 of which were from the order Nostocales. The top hits (> 96% identity) included *Atlanticothrix*, *Cyanocohniella* and *Komarekiella* species (Appendix 3).

The ML phylogenetic tree based on the BLASTN search limited to type material (plus the *G. apudmare* reference sequences and other *Goleter* sp. sequences), with the GTR+F+R6 best-fit model applied, is displayed in Figure 3. The tree partitioned

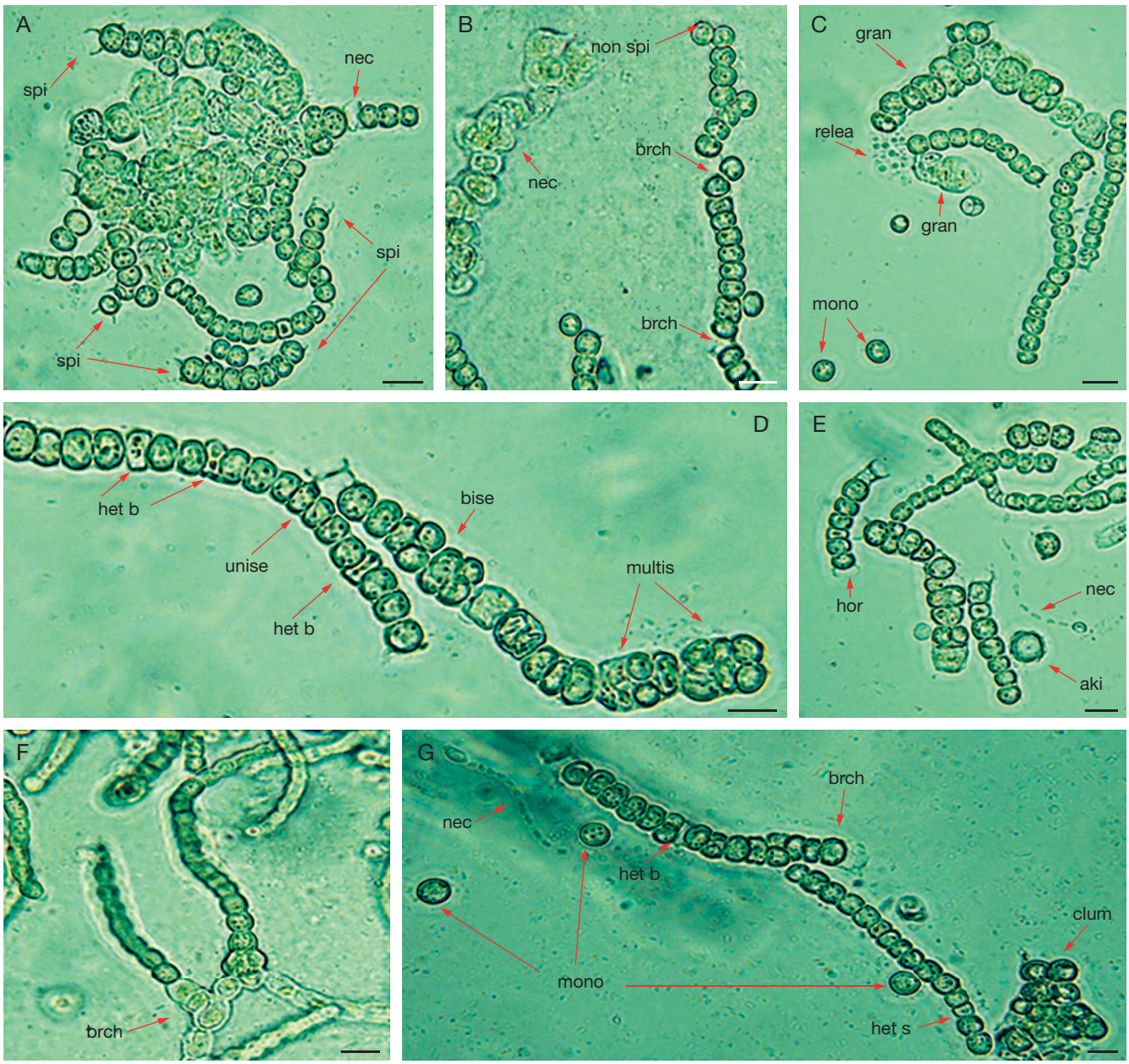


Fig. 2. — Micrographs of the isolate *Goleter* sp. in culture: **A**, older filaments featuring end cells with two corner "spines"; **B**, "spines" absent in young filaments; trichomes appear branched due to the proliferation of hormogonia derived from the main filaments; **C**, granular cell contents; **D**, filament uniseriate, biseriate or multiseriate; **E**, necridium cells occasionally found in the main filaments. Reproduction occurs via uniseriate hormogonia with two corner "spines" and bristles. Akinetes very rare; **F**, long branching filaments with prostrate main axes; **G**, trichomes often aggregate into subspherical multiseriate clumps. Heterocytes bone, shaped and spherical. Monocyte reproductive cells observed. Abbreviations: **aki**, akinete; **biser**, biseriate; **brch**, branching; **clum**, clump; **gran**, granules; **het b**, bone shaped heterocytes; **het s**, spherical shaped heterocytes; **hor**, hormogonium; **mono**, monocyte; **multis**, multiseriate; **nec**, necridium; **non spi**, "spines" absent; **relea**, released granules; **spi**, two corner "spines"; **unise**, uniseriate. Scale bars: 10 µm.

mostly according to assigned genera. The isolate clustered on the same branch as *G. apudmare* (94% ML) with *Aliinostoc* spp. and *Nodularia*, and *Cyanocohniella* and *Anabaena* forming other two sister clades. *P*-distances between the 16S rRNA gene sequences of the isolate *Goleter* sp. and its closest phylogenetic relatives are shown in Appendix 4.

### 16S-23S ITS secondary structure analysis

The ITS secondary structure of the isolate was compared to that of related *Goleter* strains (five unique sequences) identified through the 16S rRNA standard BLASTN search (Appendix 2). The D1-D1' helix, D2, D3, Box-B, Box-A, D4 and V3 regions were present in the ITS secondary structure of the isolated strain (Table 2) as described by Johansen *et al.* (2011). The D1-D1' helix of the isolate included a terminal bilateral bulge, a bilateral bulge, three unilateral bulges, and a basal clamp (Fig. 4), and was similar to that of *G. apudmare* (JN385289.1) in terms of length and shape, but significantly different to that of the *Goleter* sp. structures. The D1-D1' side loop of the isolate lacked tRNA genes for Ile and Ala, which were present in most of the

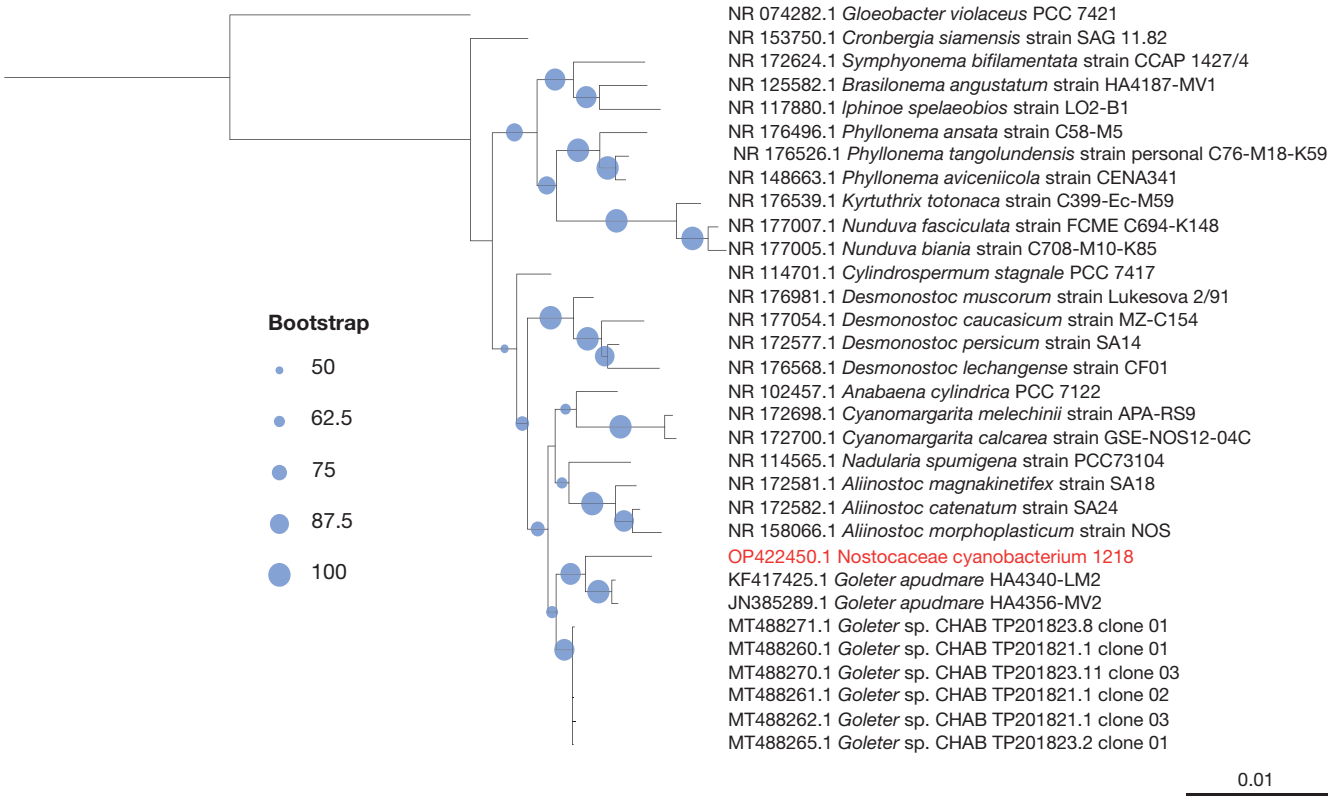


FIG. 3. — Maximum likelihood (ML) phylogenetic tree for the isolate (*Goleter* sp., in **red**) and reference strains, based on 16S rRNA gene sequences (1490 bp). *Gloeobacter violaceus* Rippka, J.B.Waterbury & Cohen-Bazire is the outgroup. Bootstrap values are shown near nodes. Scale indicates phylogenetic distance. GenBank accession numbers are indicated next to species names. For further details on sequences and BLAST results, see Appendix 3. For *p*-distances, see Appendix 4.

*Goleter* structures (Table 2). ITS sequences for *Goleter* spp., lacking tRNA genes, are not shown (see Table 2 for details). The Box-B helix of the isolate had a terminal bilateral bulge, a bilateral bulge and a basal clamp, and was similar in length and shape to the *G. apudmare* (JN385289.1) and *Goleter* sp. structures. The V3 helix of the isolate consisted of a terminal bilateral bulge, a unilateral bulge, and a basal clamp, and was significantly smaller (-8 nt) than the *Goleter* sp. structures. In *G. apudmare* lacked this region (Fig. 4; Table 2).

## DISCUSSION

Nostocales is the largest order of the phylum Cyanobacteria and contains species from diverse aquatic and terrestrial habitats (Komarek *et al.* 2014). While all Nostocalean cyanobacteria are filamentous, extraordinary morphological diversity exists in this order with respect to the presence and position of heterocytes and akinetes, filament branching, cell size and shape and various subcellular characteristics. The development of DNA sequencing technologies over the past few decades has revealed a lack of consistent correspondence between Nostocalean morphotypes and phylotypes, which has led to significant revision of the order and the description of several new taxa (Komarek *et al.* 2014). Considering these revelations, a polyphasic approach should always be undertaken when classifying new species (Komárek 2016a).

In the present study, we isolated and characterized a benthic, filamentous cyanobacterium from a freshwater pond in Golestan Province, Iran. Morphologically, this strain resembled Nostocalean member strains with branching, non-tapering filaments, and dispersed heterocytes and akinetes. In addition, molecular analysis revealed that its closest phylogenetic relative was *Goleter apudmare* (Fig. 2). *Goleter apudmare* is a terrestrial tropical species, originally isolated from the roof of a cave in Kauai, Hawaii (Miscoe *et al.* 2016). While it shares some morphological features with our isolate, it has basal akinetes and heterocytes, and tapering apices. In addition, our isolate differs in morphology from *Goleter apudmare* (Table 1) by the trichome serration, probably due to residuals of degraded dead cells, and end cells; akinete occurrence (rarely observed in the isolate, and were solitary, centrally located in, or free from filaments); heterocyst shape; filament tapering, and presence of necridia, monocyte reproductive cells and hormogonia. Unlike a monocyte, an akinete is characterized by an enveloped, thick-walled, non-motile, dormant cell formed by filamentous, heterocyst-forming cyanobacteria under the orders Nostocales and Stigonematales, which is resistant mainly to cold and desiccation. Monocytes differ from described reproductive structures including hormogonia and hormocytes (single-celled hormogonia) by the way they are produced and/or released (Berthold *et al.* 2022). While monocytes are produced and released from attached sessile apical cells through an attenuated

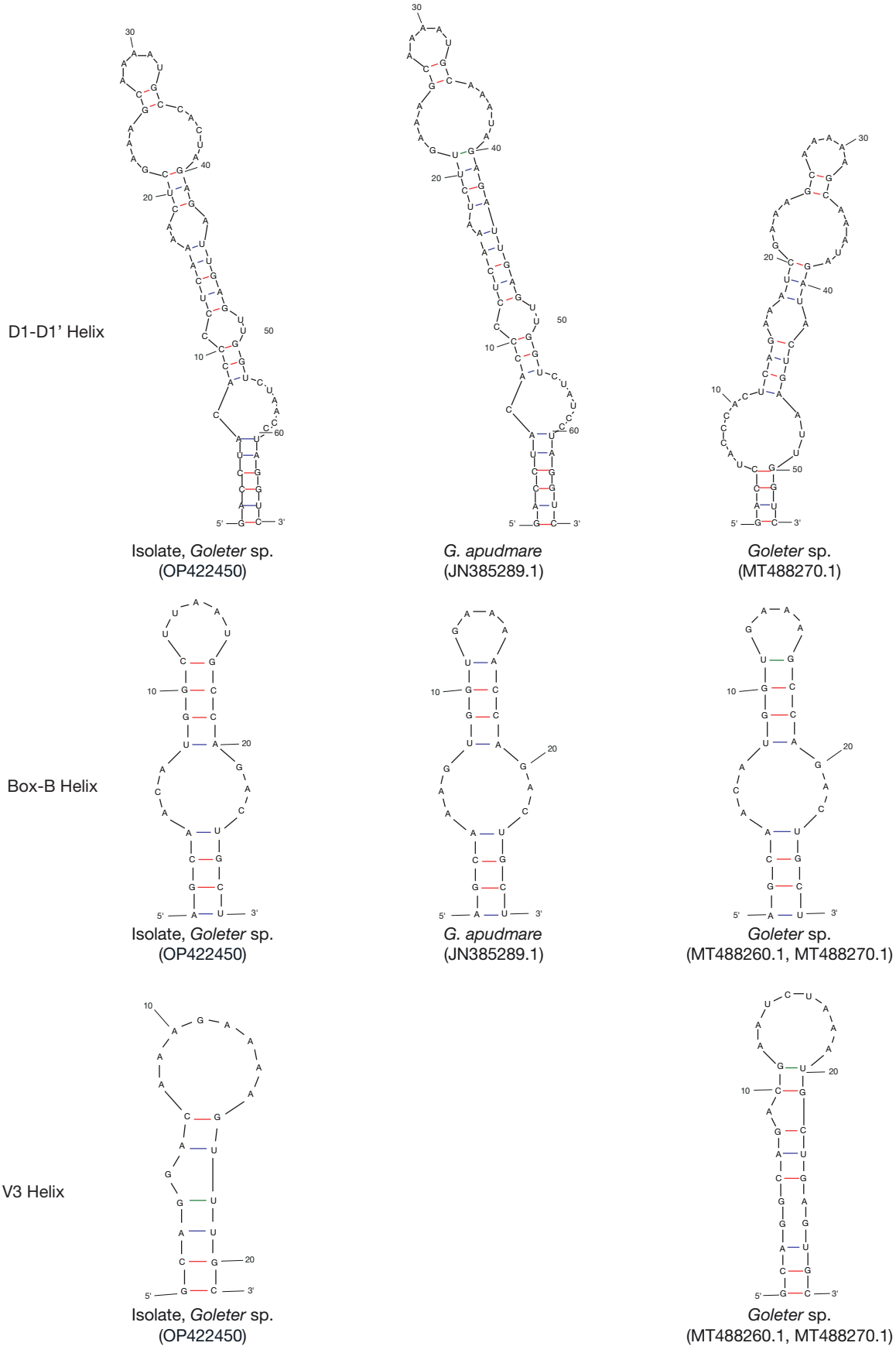


FIG. 4. — Secondary structures of the 16S-23S ITS of the isolate *Goleter* sp. and related *Goleter* sequences.

sheath, hormocytes are released after necridia or filament disintegration through an opened sheath, for instance. Moreover, reproduction by monocytes occurs through the binary fission of apical sessile cells where the apex region of the cell separates from the attached cell (Berthold *et al*. 2022).

At the molecular level, the isolate shared >97% sequence identity (99% coverage) with *Goleter* spp. by BLASTN analysis (Appendix 2). Despite significant habitat, geographical and morphological differences, the isolate and *Goleter apudmare* possess close relationship based on phylogenetic analysis. Results of the16S rRNA phylogenetic tree suggest that the isolate is a close phylogenetic relative of *G. apudmare* (*p*-distance = 0.0236, Fig. 3), belonging to the *Goleter* genus. However, it is sufficiently divergent from *G. apudmare* species, based on the taxonomic threshold (<98.7% identity) proposed by Yarza *et al*. (2014). Moreover, the cyanobacterium isolate was placed in separate branch, while two *G. apudmare* grouped together (100% ML) in the 16S rRNA phylogenetic tree.

The isolate's 16S-23S rRNA ITS secondary structure was similar to the *G. apudmare* ITS (JN385289.1) with respect to the size and shape of the D1-D1' and Box-B helixes. However, it lacked the tRNA genes for Ile and Ala compared to *G. apudmare* HA4356-MV2 (JN385288.1), while in *G. apudmare* HA4356-MV2 (JN385289.1) lacked the V3 helix (Fig. 4). Like other cyanobacterial species (Iteman *et al*. 2000; Boyer *et al*. 2001; Gugger *et al*. 2005; Finsinger *et al*. 2008), *G. apudmare* and the isolate may possess multiple ribosomal RNA operons, including variants lacking tRNA genes.

Finally, our findings contribute to increase the knowledge on diversity of the filamentous genus *Goleter*, which was previously described as a lithobiont and rock-dwelling genus. In addition, phylogeny of the Nostocaceae isolate will be better investigated in future studies in which the ITS sequence coupled with other molecular markers such as *rbc*L, *cpc*BA-IGS, and *rpo*C1, and if possible phylogenomic studies, will be performed to confirm the isolate's taxonomic position.

## Authors' contributions

Bahareh Nowruzi: original concept of paper, original draft preparation, isolation of strain, analysis of molecular data and microscope observation, construction of phylogenetic trees and ITS structures. Adriana Sturion Lorenzi: investigation, data analysis, writing – review and editing.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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# APPENDICES

Appendix 1. — Primers used to clone and sequence the 16S rRNA gene and ITS of the isolate *Goleter* sp.

| Primer name | Primer sequence (5'-3') | Purpose/target sequence | Reference |
|---|---|---|---|
| 27F1 | AGAGTTTGATCCTGGCTCAG | Cloning/sequencing 16S rRNA gene | Neilan *et al.* 1997 |
| 23S30Ra | CTTCGCCTCTGTGTGCCTAGGT | | Lepère *et al.* 2000 |
| ITS-F | TGTACACACCGCCCGTC | Cloning/sequencing 16S-23S ITS | Gkelis *et al.* 2005 |
| ITS-R | CTCTGTGTGCCTAGGTATCC | | |
| M13F | GTAAAACGACGGCCAG | Verification and sequencing of cloned PCR products | TOPO™ TA Cloning™ Kit |
| M13R | CAGGAAACAGCTATGAC | | |
| 1494R | TACGGCTACCTTGTTACGAC | Sequencing 16S rRNA gene | Neilan *et al.* 1997 |
| 979F | CGATGCAACGCGAAGAAC | | Rajaniemi *et al.* 2005 |
| 359F | GGGGAATTTTCCGCAATGGG | | Nübel *et al.* 1997 |
| 781R | ATGGGATTAGATACCCCAGTAGTC | | Nübel *et al.* 1997 |
| 544R | GCAAGCGTTATCCGGAAT | | Rajaniemi *et al.* 2005 |
| 359F | GGGGAATTTTCCGCAATGGG | | Nübel *et al.* 1997 |
| 781R | ATGGGATTAGATACCCCAGTAGTC | | Nübel *et al.* 1997 |
| 544R | GCAAGCGTTATCCGGAAT | | Rajaniemi *et al.* 2005 |
| 1092R | AAGTCCCGCAACGAGCGC | | Rajaniemi *et al.* 2005 |

Appendix 2. — BLASTN results for the 16S rRNA gene of the isolate *Goleter* sp. against the standard nucleotide database.

| Scientific name | Max score | Total score | Query cover | E value | Per. ident | Acc. len | Accession number |
|---|---|---|---|---|---|---|---|
| *Goleter apudmare* HA4356-MV2 | 2556 | 2556 | 99% | 0 | 97.84 | 1812 | JN385288.1 |
| *Goleter apudmare* HA4356-MV2 | 2555 | 2555 | 99% | 0 | 97.84 | 2025 | JN385289.1 |
| *Goleter* sp. CHAB TP201702.1 | 2575 | 2575 | 99% | 0 | 97.99 | 1876 | MT488126.1 |
| *Goleter* sp. CHAB TP201821.1 | 2569 | 2569 | 99% | 0 | 97.92 | 2099 | MT488260.1 |
| *Goleter* sp. CHAB TP201821.1 | 2564 | 2564 | 99% | 0 | 97.85 | 2077 | MT488261.1 |
| *Goleter* sp. CHAB TP201821.1 | 2547 | 2547 | 99% | 0 | 97.65 | 2099 | MT488262.1 |
| *Goleter* sp. CHAB TP201823.11 | 2575 | 2575 | 99% | 0 | 97.99 | 2099 | MT488270.1 |
| *Goleter* sp. CHAB TP201823.11 | 2575 | 2575 | 99% | 0 | 97.99 | 1878 | MT488269.1 |
| *Goleter* sp. CHAB TP201823.11 | 2575 | 2575 | 99% | 0 | 97.99 | 1878 | MT488268.1 |
| *Goleter* sp. CHAB TP201823.2 | 2575 | 2575 | 99% | 0 | 97.99 | 1877 | MT488267.1 |
| *Goleter* sp. CHAB TP201823.2 | 2569 | 2569 | 99% | 0 | 97.92 | 2098 | MT488265.1 |
| *Goleter* sp. CHAB TP201823.2 | 2564 | 2564 | 99% | 0 | 97.85 | 1877 | MT488266.1 |
| *Goleter* sp. CHAB TP201823.8 | 2575 | 2575 | 99% | 0 | 97.99 | 1878 | MT488273.1 |
| *Goleter* sp. CHAB TP201823.8 | 2569 | 2569 | 99% | 0 | 97.92 | 1878 | MT488272.1 |
| *Goleter* sp. CHAB TP201823.8 | 2564 | 2564 | 99% | 0 | 97.85 | 2099 | MT488271.1 |
| *Nodularia harveyana* BECID29 | 2447 | 2447 | 97% | 0 | 97.11 | 1450 | AJ781146.1 |
| *Trichormus* sp. SBC125 | 2492 | 2492 | 99% | 0 | 96.97 | 2204 | MW403967.1 |
| *Trichormus* sp. SBC124 | 2492 | 2492 | 99% | 0 | 96.97 | 1886 | MW403966.1 |
| Uncultured cyanobacterium | 2433 | 2433 | 97% | 0 | 96.97 | 1475 | KP762343.1 |
| Uncultured cyanobacterium | 2433 | 2433 | 97% | 0 | 96.97 | 1475 | KP762336.1 |
| *Cyanospira capsulata* CC87E | 2455 | 2455 | 98% | 0 | 96.94 | 1462 | FR774775.1 |
| Uncultured cyanobacterium | 2429 | 2429 | 97% | 0 | 96.9 | 1474 | KP762338.1 |
| Uncultured cyanobacterium | 2422 | 2422 | 97% | 0 | 96.83 | 1475 | KP762342.1 |
| Uncultured cyanobacterium | 2422 | 2422 | 97% | 0 | 96.83 | 1475 | KP762335.1 |
| Cyanobacterium UTEX B 3002 | 2418 | 2418 | 97% | 0 | 96.77 | 1451 | KP762334.1 |
| Uncultured cyanobacterium | 2416 | 2416 | 97% | 0 | 96.77 | 1474 | KP762340.1 |
| Uncultured cyanobacterium | 2416 | 2416 | 97% | 0 | 96.77 | 1474 | KP762339.1 |
| *Cyanospira capsulata* 9NAT | 2438 | 2438 | 98% | 0 | 96.73 | 1462 | FR774776.1 |
| *Cyanospira rippkae* NMBCI | 2438 | 2438 | 98% | 0 | 96.73 | 1462 | FR774768.1 |
| *Atlanticothrix silvestris* CENA590 | 2440 | 2440 | 98% | 0 | 96.73 | 2042 | MW326977.1 |
| *Atlanticothrix silvestris* CENA576 | 2440 | 2440 | 98% | 0 | 96.73 | 2042 | MW326974.1 |
| *Nodularia spumigena* UHCC 0039 | 2479 | 9889 | 100% | 0 | 96.72 | 5294286 | CP020114.1 |
| Uncultured cyanobacterium | 2418 | 2418 | 97% | 0 | 96.7 | 1475 | KP762341.1 |
| Uncultured cyanobacterium | 2418 | 2418 | 97% | 0 | 96.7 | 1475 | KP762337.1 |
| *Nodularia* sp. F81 | 2435 | 2435 | 98% | 0 | 96.67 | 1463 | AY439283.1 |
| *Cyanospira rippkae* CR86F7 | 2433 | 2433 | 98% | 0 | 96.66 | 1462 | FR774774.1 |
| *Atlanticothrix silvestris* CENA564 | 2435 | 2435 | 98% | 0 | 96.66 | 2042 | MW326973.1 |
| *Atlanticothrix silvestris* CENA585 | 2433 | 2433 | 98% | 0 | 96.66 | 2041 | MW326976.1 |
| *Nodularia harveyana* SAG 44.85 | 2431 | 2431 | 98% | 0 | 96.66 | 1461 | KM019929.1 |
| *Nodularia spumigena* CCY9414 | 2473 | 14217 | 100% | 0 | 96.65 | 5462271 | CP007203.2 |
| *Cyanocohniella* sp. SY-1-2-EE | 2433 | 2433 | 98% | 0 | 96.6 | 1465 | MT946565.1 |
| *Halotia longispora* CENA184 | 2429 | 2429 | 98% | 0 | 96.6 | 2026 | KC695875.2 |
| *Nostocaceae cyanobacterium* CCM-UFV030 | 2427 | 2427 | 98% | 0 | 96.6 | 2118 | MT708213.1 |

Appendix 2. — Continuation.

| Scientific name | Max score | Total score | Query cover | E value | Per. ident | Acc. len | Accession number |
|---|---|---|---|---|---|---|---|
| *Atlanticothrix silvestris* CENA579 | 2429 | 2429 | 98% | 0 | 96.59 | 2042 | MW326975.1 |
| *Scytonema bohneri* SAG 255.80 | 2425 | 2425 | 98% | 0 | 96.59 | 1461 | KM019923.1 |
| *Nostoc* sp. CHAB TP201728.4 | 2459 | 2459 | 99% | 0 | 96.58 | 1831 | MT488206.1 |
| *Nostoc* sp. CHAB TP201728.4 | 2459 | 2459 | 99% | 0 | 96.58 | 2149 | MT488205.1 |
| *Nodularia* sp. CHAB TP201734.3 | 2459 | 2459 | 99% | 0 | 96.57 | 1828 | MT488224.1 |
| *Nodularia harveyana* Bo53 | 2459 | 2459 | 99% | 0 | 96.57 | 1564 | AJ781143.1 |
| *Nostoc* sp. ACSSI 329 | 2427 | 2427 | 98% | 0 | 96.53 | 1466 | MT425943.1 |

Appendix 3. — BLASTN results for the 16S rRNA gene of the isolate *Goleter* sp. against type strains. *, not listed as type strain in GenBank.

| Scientific name | Max score | Total score | Query cover | E value | Per. ident | Acc. len | Accession number |
|---|---|---|---|---|---|---|---|
| *Goleter apudmare** | 2457 | 2457 | 99% | 0 | 97.8 | 2025 | JN385289.1 |
| *Atlanticothrix silvestris* | 2350 | 2350 | 95% | 0 | 96.6 | 1414 | NR_172568.1 |
| *Cyanocohniella crotaloides* | 2403 | 2403 | 98% | 0 | 96.3 | 1500 | NR_176548.1 |
| *Cyanocohniella rudolphia* | 2414 | 2414 | 99% | 0 | 96.2 | 1560 | NR_176566.1 |
| *Komarekiella globosa* | 2362 | 2362 | 97% | 0 | 96.2 | 1444 | NR_176586.1 |
| *Nodularia spumigena* | 2399 | 2399 | 99% | 0 | 95.8 | 1517 | NR_114565.1 |
| *Dendronalium phyllosphericum* | 2276 | 2276 | 95% | 0 | 95.7 | 1412 | NR_172569.1 |
| *Desmonostoc muscorum* | 2350 | 2350 | 98% | 0 | 95.6 | 1462 | NR_176981.1 |
| *Cylindrospermum stagnale* PCC 7417 | 2316 | 2316 | 97% | 0 | 95.6 | 1444 | NR_114701.1 |
| *Komarekiella atlantica* | 2372 | 2372 | 99% | 0 | 95.6 | 1479 | NR_172689.1 |
| *Aliinostoc catenatum* | 2364 | 2364 | 99% | 0 | 95.4 | 1483 | NR_172582.1 |
| *Aliinostoc morphoplasticum* | 2351 | 2351 | 99% | 0 | 95.3 | 1482 | NR_158066.1 |
| *Desmonostoc persicum* | 2331 | 2331 | 98% | 0 | 95.3 | 1546 | NR_172577.1 |
| *Anabaena cylindrica* PCC 7122 | 2333 | 2333 | 99% | 0 | 95.2 | 1477 | NR_102457.1 |
| *Constrictifilum karadense* | 2305 | 2305 | 98% | 0 | 95 | 1468 | NR_172623.1 |
| *Amazonocrinis nigriterrae* | 2320 | 2320 | 99% | 0 | 94.9 | 1482 | NR_172622.1 |
| *Kryptousia microlepis* | 2278 | 2278 | 98% | 0 | 94.8 | 1460 | NR_157979.1 |
| *Aliinostoc magnakinetifex* | 2287 | 2287 | 98% | 0 | 94.7 | 1471 | NR_172581.1 |
| *Kryptousia macronema* | 2252 | 2252 | 98% | 0 | 94.5 | 1457 | NR_157980.1 |
| *Nostoc punctiforme* PCC 73102 | 2278 | 2278 | 100% | 0 | 94.3 | 1489 | NR_074317.1 |
| *Desmonostoc lechangense* | 2102 | 2102 | 92% | 0 | 94.2 | 1385 | NR_176568.1 |
| *Desmonostoc caucasicum* | 2209 | 2209 | 98% | 0 | 94 | 1457 | NR_177054.1 |
| *Dapisostemon apicaliramis* | 2202 | 2202 | 97% | 0 | 94 | 1455 | NR_172678.1 |
| *Desikacharya nostocoides* | 2224 | 2224 | 98% | 0 | 94 | 1560 | NR_176532.1 |
| *Nostoc favosum* | 2235 | 2235 | 99% | 0 | 93.8 | 1487 | NR_176571.1 |
| *Pseudoaliinostoc constrictum* | 2226 | 2226 | 99% | 0 | 93.8 | 1479 | NR_172583.1 |
| *Cronbergia siamensis* | 2193 | 2193 | 98% | 0 | 93.8 | 1461 | NR_153750.1 |
| *Cyanomargarita melechinii* | 2202 | 2202 | 98% | 0 | 93.6 | 1473 | NR_172698.1 |
| *Cyanomargarita calcarea* | 2193 | 2193 | 99% | 0 | 93.4 | 1479 | NR_172700.1 |
| *Phyllonema tangolundensis* | 2180 | 2180 | 99% | 0 | 93.2 | 1481 | NR_176526.1 |
| *Nostoc oromo* | 2169 | 2169 | 99% | 0 | 92.9 | 1489 | NR_172703.1 |
| *Phyllonema ansata* | 2082 | 2082 | 96% | 0 | 92.9 | 1500 | NR_176496.1 |
| *Phyllonema aviceniicola* | 2028 | 2028 | 95% | 0 | 92.6 | 1414 | NR_148663.1 |
| *Symphyonema bifilamentata* | 2117 | 2117 | 99% | 0 | 92.5 | 1475 | NR_172624.1 |
| *Aliterella antarctica* | 2115 | 2115 | 99% | 0 | 92.4 | 1483 | NR_151904.1 |
| *Wilmottia stricta* | 2093 | 2093 | 99% | 0 | 92.2 | 1482 | NR_177020.1 |
| *Iphinoe spelaeobios* | 2034 | 2034 | 97% | 0 | 92 | 1446 | NR_117880.1 |
| *Brasilonema angustatum* | 2071 | 2071 | 99% | 0 | 92 | 1479 | NR_125582.1 |
| *Nunduva fasciculata* | 2067 | 2067 | 99% | 0 | 91.9 | 1478 | NR_177007.1 |
| *Gloeocapsopsis dulcis* | 2073 | 2073 | 99% | 0 | 91.9 | 1490 | NR_172677.2 |
| *Kyrtuthrix totonaca* | 2025 | 2025 | 97% | 0 | 91.9 | 1500 | NR_176539.1 |
| *Aliterella chasmolithica* | 2034 | 2034 | 98% | 0 | 91.8 | 1500 | NR_176549.1 |
| *Symplocastrum torsivum* | 2032 | 2032 | 98% | 0 | 91.8 | 1463 | NR_172658.1 |
| *Gloeocapsopsis crepidinum* | 2061 | 2061 | 99% | 0 | 91.8 | 1483 | NR_172660.2 |
| *Nunduva biania* | 2060 | 2060 | 99% | 0 | 91.7 | 1481 | NR_177005.1 |
| *Potamolinea magna* | 2058 | 2058 | 99% | 0 | 91.7 | 1485 | NR_151862.1 |
| *Potamolinea aerugineo-caerulea* | 2058 | 2058 | 99% | 0 | 91.7 | 1485 | NR_151861.1 |
| *Aliterella atlantica* | 2026 | 2026 | 98% | 0 | 91.5 | 1546 | NR_177002.1 |
| *Pleurocapsa minor* | 2045 | 2045 | 99% | 0 | 91.5 | 1490 | NR_172667.1 |
| *Arizonema commune* | 2030 | 2030 | 99% | 0 | 91.3 | 1491 | NR_176554.1 |
| *Wilmottia koreana* | 2019 | 2019 | 99% | 0 | 91.2 | 1488 | NR_172594.1 |

Appendix 4. — *P*-distances between the 16S rRNA gene sequences of the isolate *Goleter* sp. and its closest phylogenetic relatives. GenBank accession numbers are shown in **parentheses**.

| Sequence 1 | Sequence 2 | *p*-distance |
|---|---|---|
| *Goleter* sp. (OP422450, nt 121-1611) | *Goleter apudmare* (JN385289.1) | 0.0236 |
| | *Cyanocohniella crotaloides* (NR_176548.1) | 0.0374 |
| | *Cyanocohniella rudolphia* (NR_176566.1) | 0.0412 |
| | *Nodularia spumigena* (NR_114565.1) | 0.0437 |
| | *Aliinostoc catenatum* (NR_172582.1) | 0.0457 |
| | *Aliinostoc morphoplasticum* (NR_158066.1) | 0.0484 |
| | *Aliinostoc magnakinetifex* (NR_172581.1) | 0.0556 |

Appendix 5. — Total length in base-pairs (385 bp) of the *Goleter* sp. ITS sequence and location of each motif. **Dark blue** corresponds to the 16S rRNA sequence.

| | D1-D1' helix | Spacer + D2 + spacer | D3 | tRNAIle gene | tRNAAla gene | Box-B | Post Box-B spacer | Box-A | D4 | V3 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Goleter* sp. (OP422450) | 65 | 35 | 4 | – | – | 27 | 17 | 11 | 8 | 21 |
| Color matching | Red | Yellow | Pink | – | – | Green | – | Blue pale | Gray | Olive green |

CCGAACCATGAAAAATATTATAGTTTGAAAGAAGACAGCAAATATTCCGCGTCAAATCAATAAACTAACTAGACTGAAGT
TAAAAAAGCAGTCAAAAGAGCTAAAAACAAACAATTCAAAACGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGGCGG
TATGCTTAACACATGCAAGTCGAACGGTCTCTTCGGAGATAGTGGGCGGACGGGTGAGTAACGCGTGAGAATCTAGCTTCAG
GTTCGGGACAACCACTGGAAACGGTGGCTAATACCGAATGTGCCGAGAGGTGAAAGGCTTGCTGCCTGAAGATGAGCTCGC
CCGTCTGATTAGCTAGTTGGTGGGGTAAAAGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAAAGCCTGACG
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GTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTTTTAGTTGCCAG
CATTCAGTTGGGCACTCTAAAGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGCCCCT
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GAATTCGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGAAGCTGGTCACGCCCGAAGTCATTACCCCAACTTTTCG
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CTAGGTCGGTCGAGTATTGGTAAAAGCTTTCAAAGTATCTCTGGTTCAGTTTATAAATAGTTACAACCAAATTGAGCG
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GCAGGACAAAGAAAAGTTTGCAGGTGAAACACCAAATGTATTGTGGTCAAGCTAATAAGGGCTAACGGTTGGATACCTAGGC