



Article

Alkalinema pantanalense and Roholtiella edaphica (Cyanobacteria): Two New Species Records for Egypt

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Abstract

Our current knowledge of the cyanobacterial diversity in Egypt is still underestimated During our routine study on Egyptian cyanobacteria, two interesting and morphologically cryptic strains were isolated from streams of Bahr Yussef and Qarun Lake, one of the oldest lakes in the world, located at the Faiyum depression, Egypt. We applied the polyphasic approaches, combining the state-of-the-art morphotaxonomy, 16S rRNA gene phylogenies, and ecological preferences to precisely unravel the taxonomic positions of these two cyanobacterial strains. Based on a combination of their morphotaxonomic traits and 16S rRNA phylogenetic assessment, we identified them as Alkalinema pantanalense (Leptolyngbyaceae, Leptolyngbyales) and Roholtiella edaphica (Nostocaceae, Nostocales). Both species are considered new cyanobacterial records for Egypt and the African continent based on the available literature. From an ecological standpoint, both species are eutraphentic, where they could tolerate relatively elevated concentrations of NO₃⁻, NH₄⁺ (in particular for R. edaphica), and silicates, reflecting eutrophication signs in the ecosystems they colonize. This study adds to the limited molecular information available on the Egyptian cyanobacteria, and also highlights the need for re-investigation of Egyptian cyanobacteria, using polyphasic approaches, to better understand their taxonomy and ecology.

Keywords: cyanoprokaryotes; Egypt; new records; 16S rRNA; modern taxonomy



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1. Introduction

Cyanobacteria are autotrophic gram-negative bacteria with heterogeneous genera and species. Cyanobacteria inhabit diverse ecosystems, ranging from freshwater to marine and even extreme terrestrial environments [1]. Morphological diagnostic traits and lifecycle stages have long been used to clarify their taxonomic positions, although these criteria are currently insufficient to distinguish species complexes and cryptic species and genera [2–4]. Over the past decades, numerous studies addressed the necessity of polyphasic approaches, combining morphological, ecological, and eco-physiological traits to precisely identify different species and infraspecies of cyanobacteria [5–8]. With the aid of sequencing 16S rRNA and 16S–23S rRNA internal transcribed spacer (ITS) genes, as well as the application of modern taxonomy standards for describing novel species, rapid changes in cyanobacterial taxonomy have been taking place [9–12].

The family Leptolyngbyaceae is distinguished by having thin trichomes, 1.5–2.5 (–4.5) μ m wide, and cylindrical cells with facultatively constricted cross-walls [10]. Applying the

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polyphasic approach, several new and cryptic genera, which are morphologically very similar to the genus *Leptolyngbya* Anagnostidis & Komárek, have been detected. Among them, the genus *Alkalinema* Vieira Vaz et al. was described in alkaline and saline wetlands in the Pantanal, Brazil [13]. Taxonomically, *Alkalinema* is a monotypic genus with the type species *Alkalinema pantanalense Vieira Vaz* et al., which is characterized by having trichomes which are frequently organized in ornate (interwoven) mats in liquid medium. The color of the culture ranges from red to brown. Sheath is usually absent, but the trichomes have diffluent mucilages and are non-motile. Cell content is homogeneous, and the shape ranges from isodiametric to rectangular, 2.0–4.1 μ m long \times 1.7–2.2 μ m broad. Apical cells are rounded–conical or narrowed. Reproduction usually occurs by hormogonia formation.

While conducting their broader survey on the hidden diversity of terrestrial cyanobacteria, Bohunicka et al. [14] discovered the new genus *Roholtiella* Bohunická, Pietrasiak & J.R. Johansen (Nostocaceae, Nostocales) from soil localities in the USA, Russia, and Czech Republic. Morphologically, the genus *Roholtiella* shares some taxonomic traits with the genus *Calothrix* C.Agardh ex Bornet & Flahault, but it is still distinguished by having a series of arthrospores and existing without a tapering terminal hair. Ecologically, *Roholtiella* is usually found in fresh water and soils. Phylogenetically, *Roholtiella* is also close to the genus *Calochaete* Hauer, Bohunická & Mühlsteinová, but they are still different in the conserved structures of the 16S–23S ITS regions. So far, there are only six taxonomically accepted species of the genus *Roholtiella* [15]: *R. bashkiriorum* Gaysina & Bohunická, *R. edaphica* Bohunická & Lukesová, *R. fluviatilis* J.R. Johansen & Gaysina, *R. mojavensis* N. Pietrasiak & J.R. Johansen [14], *R. mixta* Abdullin & A.A. Gontcharov [16], and *R. volcanic* Gaysina et al. [17].

Previous studies of cyanobacteria in Egypt have been largely depended on classical morphotaxonomy systems. These studies have documented the presence of various cyanobacterial species in diverse habitats, including the Nile River and its delta [18-20]. However, this traditional taxonomic approach most often overlooks the genetic diversity and ecological preferences of cyanobacteria, leading to an incomplete understanding of their accurate taxonomic positions and distribution. Recent research on Egyptian cyanobacteria has uncovered the presence of some species new to science, and has highlighted the need for integrating molecular techniques, such as DNA sequencing and phylogenetic analysis, and modern taxonomy standards to obtain a more accurate and comprehensive picture of the diversity of cyanobacteria [1,21,22]. For instance, the recent study conducted by Hentschke et al. [23] on a filamentous cyanobacterial strain that was isolated from a benthic mat in an irrigation canal in the Nile discovered the novel species *Pegethrix nilien*sis G.S. Hentschke. Qasem and other co-workers [24] also discovered the new cyanobacterial species Desikacharya aegyptiaca Qasem, El-Gamal, Mahfouz, A.A. Saber & Salah El Din from the Egyptian soils. Generally, taxonomic knowledge about Egyptian, and also African [25–27], cyanobacteria is still understudied [22,28]. Komárek [28] pinpointed that the diversity of cyanobacteria in North Africa includes several cryptic species complexes, and these taxa require more detailed investigations, including genetic, ecophysiological, and phenotypic taxonomy systems.

This paper documents the existence of *Alkalinema pantanalense* and *Roholtiella edaphica* as new cyanobacterial species records for Egypt in streams of Bahr Yussef and Qarun Lake, respectively, in the Faiyum depression, Egypt. Morphotaxonomy, ecology, and 16S rRNA phylogeny of both species are provided.

2. Materials and Methods

2.1. Sampling and Isolation of Cyanobacterial Strains

Our two strains were isolated from water samples collected in two different *loci* in the El-Faiyum governorate, Egypt (Figure 1). The strain *Alkalinema pantanalense* RMFY20

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was isolated on August 2020 from Bahr Yussef (29°16′32.5″ N, 30°49′21.6″ E). Bahr Yussef is a small canal which feeds water northwards into the Faiyum Oasis, parallel with the Nile. The second strain, *Roholtiella edaphica* AR5, was isolated in July 2021 from the eastern sector of Qarun Lake, near the outlet of El-Batts drain (29°30′14.0″ N, 30°50′36.6″ E). Qarun Lake is a historical lake located in the Faiyum Oasis, ~80 km southwest of Cairo, which is nowadays just a small portion of its previous size. Each cyanobacterial strain was isolated using the streaking technique from the cyanobacterial and microalgal cultures grown from each ecosystem. A culture flask containing MBL medium [29] was used for the cultivation of *A. pantanalense* RMFY20 at 25 \pm 1 °C, while BG-110 medium [30] was used for *R. edaphica* AR5 at 29 \pm 1 °C. Both strains were kept under a 16:8 h light:dark photocycle using white fluorescent light (40 µmol photons m⁻² s⁻¹).

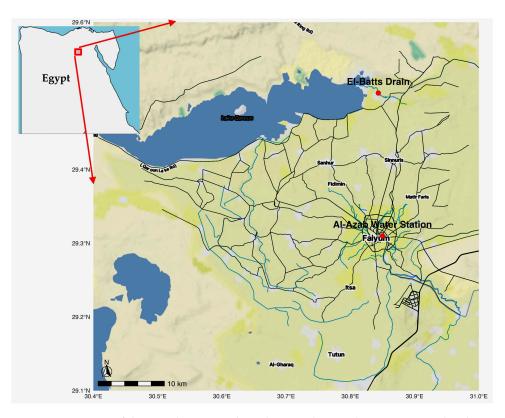


Figure 1. Locations of the sampling sites where the cyanobacterial strains were isolated.

2.2. Hydrochemical Analyses

The seasonal physical and chemical parameters of water samples of the two ecosystems studied were measured following the protocols adapted by the American Public Health Association Standard Methods [31,32]. Water conductivity and pH were measured in situ. Ammonium (NH₄⁺) concentrations were determined using the indophenol method following the method adapted by Bremner and Shaw [33]. Nitrite (NO₂⁻) was assessed using a colorimetric method [34]. Nitrate (NO₃⁻) was measured according to the protocol described by Mullin and Riley [35]. Orthophosphates (PO₄³⁻) were measured following the stannous chloride method. Silicate was estimated using the molybdosilicate method [36].

2.3. DNA Extraction, PCR Amplification, Sequencing, and Phylogenetic Analysis

DNA extraction and PCR amplification were carried out using the primers WAW8F (5'-AGAGTTTGATCCTGGCTCAG-3') and WAW23SR (5'-TTTGCGGCCGCTGTGTGCCTAGG TATCC-3') to amplify the 16S rRNA gene, as described by Casamatta et al. [37]. For phylogenetic assessment, BLAST searches were performed using all of the 16S rRNA sequences generated against the NCBI core nucleotide database. The phylogenetic relationships of

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the studied strains were inferred using Bayesian inference implemented in MrBayes 3.2.7a arm. The nucleotide substitution model GTR+I+G (General Time Reversible model with a proportion of invariable sites and gamma-distributed rate heterogeneity) [38] was selected. Two independent runs were performed, each with four Markov chains (one cold, three heated) for 5,000,000 generations, sampling every 1000 generations. A burn-in fraction of 25% was applied, and convergence was assessed by examining the average standard deviation of split frequencies (<0.01) and the potential scale reduction factor (PSRF \approx 1.0). A 50% majority-rule consensus tree was generated with posterior probabilities (PP) mapped onto the nodes. This was applied as the best-fit model of nucleotide substitution, and 1000 bootstrap replications were used to assess how robust the inferred phylogenetic connections were. A total of 16S rRNA gene sequences of the cyanobacterial strains were submitted to the NCBI GenBank database under accession numbers OR469049 and OR610310 for *Alkalinema pantanalense* RMFY20 and *Roholtiella edaphica* AR5, respectively.

2.4. Morphological Analyses

For morphological analyses, each strain was examined and microphotographed using a BEL® photonics biological microscope (BEL® Engineering, Monza, Italy) fitted with a Canon Powershot G12 digital camera. The specimens were identified following the relevant taxonomic characteristics in Vieira Vaz et al. [13] for *Alkalinema pantanalense* RMFY20, and in Bohunická et al. [14] for *Roholtiella edaphica* AR5. In each cyanobacterial strain, 20–30 measurements of each taxonomic characteristic were carried out at various positions of the slides prepared.

3. Results

3.1. Hydrochemical Characterization

In general, and during the study period, Qarun Lake water contained a higher concentration of NH $_4$ ⁺ ions (750 µg.L $^{-1}$) than Bahr Yussef (89 µg.L $^{-1}$). However, average concentrations of silicates (1900 µg.L $^{-1}$) and PO $_4$ ^{3 $^{-1}$} (200 µg.L $^{-1}$) in Bahr Yussef were distinctly higher than those in Qarun Lake (1206 and1531 µg.L $^{-1}$, respectively) (Figure 2). Both ecosystems were in general characterized by having alkaline water, with an average pH value of 8.5 at Qarun Lake and 7.8 at Bahr Yussef. The average water temperature gradients were 33.1 °C at Qarun Lake and 30 °C for Bahr Yussef, reflecting the warm conditions typical for these aquatic ecosystems.

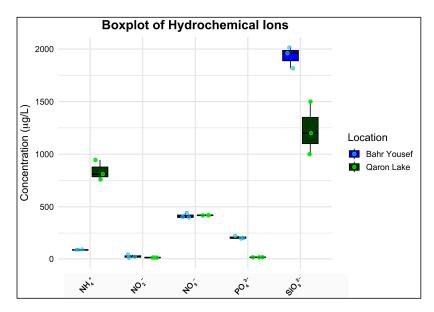


Figure 2. Boxplots showing average concentrations of the hydrochemical variables of Bahr Yussef and Qarun Lake in the present study.

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3.2. Taxonomic Descriptions

3.2.1. Alkalinema Pantanalense Vieira Vaz et al. [13] (Figure 3a-h)

Description: Trichomes grow free-floating in liquid medium with reddish to brownish color. Trichomes solitary or organized in interwoven mats. Each trichome has a thin sheath. Cells are isodiametric or longer, with a homogeneous content and reddish to brownish, 1.5–2.5 μ m wide \times 2.0–3.0 μ m long. End cell rounded to narrowly short conical. Reproduction only occurs by hormogonia.

Distribution in Egypt: This is the first record of this cyanobacterial species in Egypt where it was found in Bahr Yussef, the Faiyum depression, Egypt.

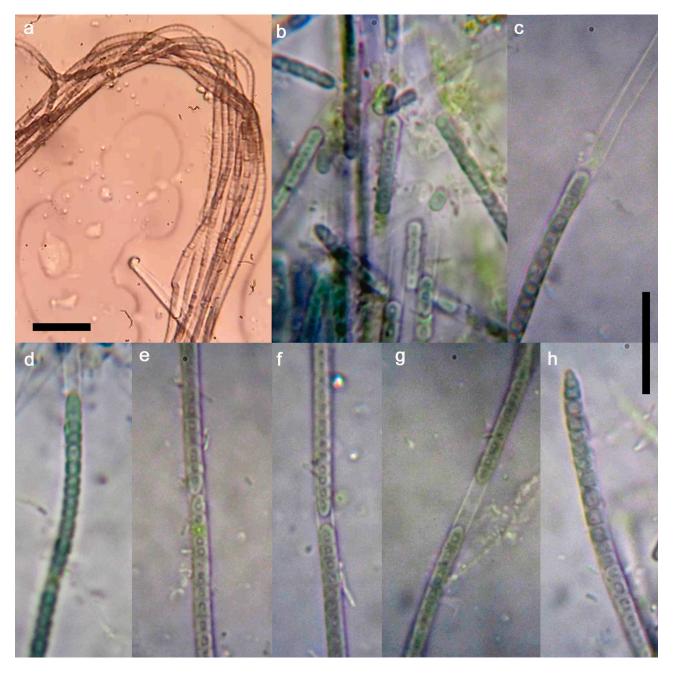


Figure 3. Light micrographs of *Alkalinema pantanalense*: (a) macroscopic interwoven mats growing in liquid medium, (b) details of trichomes and sheaths, (c) trichome with rounded end cell, (d,h) trichomes with narrowly short conical end cells, (e-g) hormogonia formation and trichome disintegration. Scale bars: $10 \, \mu m$.

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3.2.2. Roholtiella edaphica Bohunická & Lukesová [14] (Figure 4a-s)

Description: Thallus olive-green to brown-olive, with short-to-long heteropolar filaments which are usually single and rarely false-branched. Sheath colorless or slightly brown, thin and firm, sometimes widened in older filaments, closed at the ends or becoming diffluent towards the ends, or open when the arthrospores are released. Trichomes 5.5–11.0 μm wide, gradually tapered and constricted at cross-walls, sometimes swollen at the base where the widest cells are basal or rarely intercalary. Cells are generally shorter than wide or sometimes slightly longer than wide, barrel-shaped to nearly spherical, or spherical–compressed, 2.5–9.0 μm long. Cell content is smooth or finely granulated. End cells conical or conical rounded, 4.0–7.0 μm long \times 3.0–5.5 μm wide. Heterocytes, both intercalary and terminal, are pale yellow or yellow. Intercalary heterocytes are usually barrel-shaped and shorter than wide, but terminal heterocytes are hemispherical to spherical, 3.0–8.5 μm long \times 5.0–10.0 μm wide. Reproduction occurs by the formation of short hormogonia and arthrospores. Arthrospores are usually released from the end of the filaments by dissociation, 4.5–9.0 μm long \times 6.0–11.5 μm wide.

Distribution in Egypt: The finding of this cyanobacterial species in Qarun Lake represents the first documented instance of its existence in Egypt.

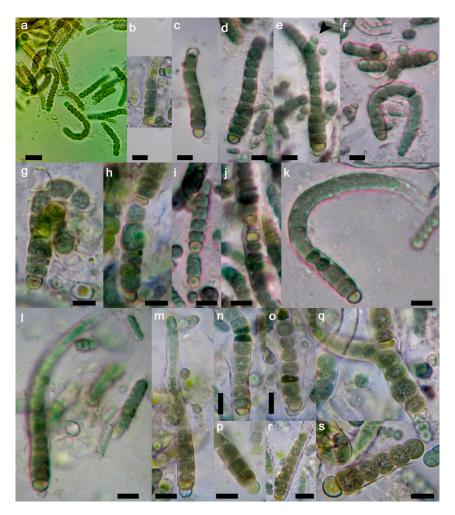


Figure 4. Light micrographs of *Roholtiella edaphica*: (a–c) hormogonia, (d–g) young filaments with basal heterocytes. Note the beginning of false branching (arrowhead) and swollen bases in (e). (h–j) Filaments with intercalary heterocytes, (k–p,r) details of tapered mature filaments. Note the diffluent sheath at the filament end (m), and the firm, widened sheath at the filament base (o). (q) Mature filament with a false branching at the intercalary compressed heterocyte, (s) formation and releasing of arthrospores. Scale bars: $10 \, \mu m$, except (a) $20 \, \mu m$.

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3.3. Phylogenetic Assessment

The Bayesian phylogenetic tree (Figure 5) based on the 16S rRNA gene sequences resolved the studied taxa into well-supported clades. The GTR+I+G model provided a robust fit to the data, and most nodes were supported by high posterior probabilities (\geq 0.95).

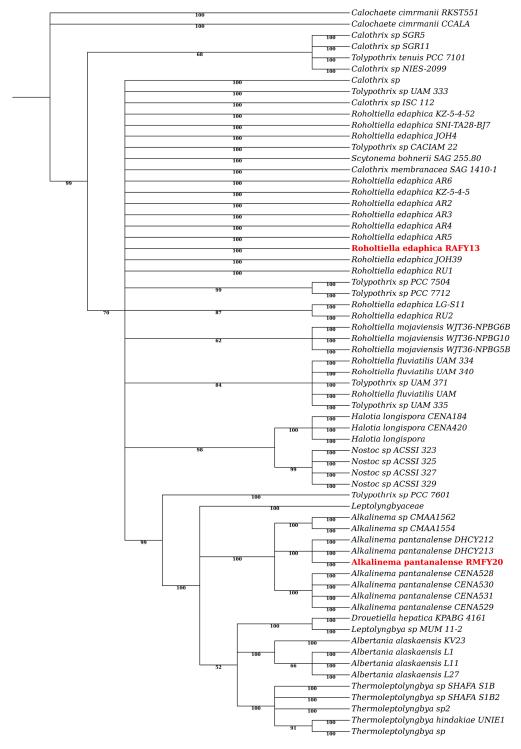


Figure 5. Bayesian consensus phylogenetic tree of the studied cyanobacterial strains based on 16S rRNA gene sequences. The analysis was performed in MrBayes under the GTR+I+G substitution model, with two runs of four chains each for 5,000,000 generations. Posterior probabilities (≥0.95) are shown at the nodes. The newly sequenced strains *Roholtiella edaphica* RAFY13 and *Alkalinema pantanalense* RMFY20 are highlighted in bold red color.

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A sample was taken every 1000 generations in two simultaneous runs of 5 million generations, with the first 25% being destroyed as burn-in. Strong phylogenetic resolution was shown by the consensus tree's high posterior probability support across the majority of internal nodes, with values ranging from 95 to 100% for most branches.

With posterior probability of 100% (PP = 100), the isolate *Roholtiella edaphica* RAFY13 grouped securely within the *Roholtiella* clade alongside reference strains (*R. edaphica* AR2–AR6, AR39, AR45, JOH4, JOH39, and RU1). In a similar manner, *Alkalinema pantanalense* RMFY20 was assigned to the *Alkalinema* lineage, forming a group comprising reference strains (DHCY212 and DHCY213) and type strains (*A. pantanalense* CENA528–CENA531) that had 100% posterior support. These positions provide strong phylogenetic evidence for the two isolates affiliated with the genera *Roholtiella* and *Alkalinema*, respectively, and validate their identities as *R. edaphica* and *A. pantanalense*. In line with earlier taxonomic frameworks, the tree also retrieved the more general relationships between the groups Nostocaceae, Tolypothrichaceae, and Leptolyngbyaceae.

Overall, the phylogeny confirms the placement of the newly isolated strains within their respective genera and supports their affiliation as new records for Egypt.

To complement the tree topology in Figure 6, a heatmap of patristic distances (substitutions per site) was generated using branch length information from the consensus tree. This matrix representation highlighted the relative genetic divergence among taxa, with closely related strains forming blocks of low distance values (blue), while more distantly related taxa exhibited higher distances (yellow-to-green). Together, the Bayesian phylogeny and patristic distance heatmap provide strong evidence for the accurate placement of the studied isolates within their respective genera and reveal the degree of evolutionary divergence among the sampled cyanobacteria.

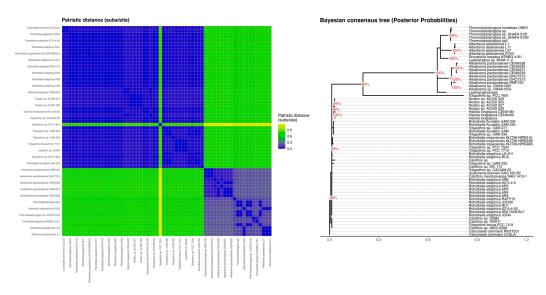


Figure 6. Comparative visualization of phylogenetic relationships among cyanobacterial strains. (Left) Heatmap of patristic distances (substitutions per site) based on branch lengths of the Bayesian consensus tree, with warmer colors (yellow–green) indicating greater divergence and cooler colors (blue–purple) reflecting closer relationships. (Right) Bayesian consensus phylogenetic tree inferred from 16S rRNA gene sequences under the GTR+I+G substitution model in MrBayes (5,000,000 generations, two runs, four chains). Posterior probabilities (≥95%) are shown at nodes. The newly sequenced strains *Roholtiella edaphica* RAFY13 and *Alkalinema pantanalense* RMFY20 are highlighted in bold red color.

To exclude potential affinity with *Calochaete*, we included representative *Calochaete* 16S sequences in our phylogeny. Bayesian inference grouped the isolate firmly within *Roholtiella* (posterior probability = 1.00), consistent with morphology and supporting its

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identification as *Roholtiella*. We note that 16S–23S ITS would provide additional resolution, and we plan to pursue it in future work.

4. Discussion

Our strain of Alkalinema pantanalense was placed in a well-supported cluster mainly composed of A. pantanalense sequences based on the 16S rRNA phylogenetic assessment. Morphometric diagnostic features of the Egyptian A. pantanalense strain examined also coincide with the original description provided by Vieira Vaz et al. [13]: thallus morphology, cell outline and dimensions, and mode of reproduction. However, our A. pantanalense strain is distinguished by the presence of a sheath around each trichome. This taxonomic trait has not been reported in the description of the holotype [13]. Supporting our findings, dela Cerna and Geraldino [39] isolated six sheathed strains of A. pantanalense from a hot spring in Negros Oriental (Philippines), and confirmed their identifications using an integrated approach of morphological and molecular data. During their phylogenetic and taxonomic study on the diversity of homocytous cyanobacteria, Genuário et al. [40] also documented the presence of a sheath in trichomes of the strain A. pantanalense CMAA1554 isolated from Amazonian rivers. We think that the presence and/or absence of the sheath in A. pantanalense is a facultative characteristic belonging to the environmental conditions, and its presence in the Egyptian strain is a type of phenotypic adaptation to the environment 'ecomorphotype'. From an ecological standpoint, our A. pantanalense was isolated from the alkaline nutrient-rich freshwater stream of Bahr Yussef. In general, it has been reported that A. pantanalense can be found in a wide ecological niche, i.e., saline–alkaline Pantanal wetlands in Brazil [13], phytoplanktonic or epilithic species living on gravel in freshwaters in South Korea, China, Brazil, and Sri Lanka [40–43], and even in cryoconite holes in Antarctica [44], indicating its high adaptive environmental capability and extensive ecological distribution. Based on the available literature, this study provides the first record of A. pantanalense in Egypt and the African continent.

Given that the *Roholtiella edaphica* clade includes the Egyptian strain, with typical *R. edaphica* morphology, and other established strains of the species, we, therefore, maintain the conservative identification of the Egyptian material as *R. edaphica*. From an ecological point of view, this study pinpointed that R. edaphica is frequent in the planktonic assemblages of the alkaline nutrient-rich Qarun Lake. However, this species was first described from Sahara soil in the USA by Bohunicka et al. [14], and future research studies reported its presence in the terrestrial cyanobacterial diversity of Eurasia [45]. This study highlights that *R. edaphica* has a high degree of ecological plasticity and resilience to changing environmental conditions, where it can occur in mineral- and nutrient-rich ecosystems such as Qarun Lake, in addition to its well-known ecological preferences for the soils. Due to its specific reproductive structures that can resist prolonged desiccation, we think that this cyanobacterial species can be ecologically adapted to these harsh environmental conditions.

We think that both *A. pantanalense* and *R. edaphica* have been transferred from the Sahara Desert across the Atlantic Ocean into both North and South America. Supporting our hypothesis, a recent study conducted by Johansen et al. [46] described the genetically close cyanobacterial species *Pseudoacaryochloris cystiformans* Jusko & J.R. Johansen and *P. abyssiniae* Melaku Mesfin & J.R. Johansen from the San Nicolas Island and Ethiopia, respectively, supporting the hypothesis of the dispersal of cyanobacteria from arid soils in Africa and their establishment in the Americas, followed by genetic lineage separation in the immigrant populations. Wind is indeed the most likely vector for dispersal of the soil microbes [47], and cyanobacteria have been emphasized to be present in atmospheric samples [48]. It has been hypothesized that dust particles originating in the Sahara Desert in Africa have been found in both North and South America [49,50]. The same conclusion

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can be applied for cyanobacteria, where they could make a similar journey across the Atlantic Ocean [46]. Conclusively, and based on our integrative polyphasic study and the available literature, both *A. pantanalense* and *R. edaphica* are considered new cyanobacterial species for Egypt and Africa.

5. Conclusions

In conclusion, reporting the two cyanobacterial species *Alkalinema pantanalense* and *Roholtiella edaphica* in Egypt not only partially contributed to our better understanding of the hidden and cryptic diversity of cyanobacteria in Egypt and Africa, but also expanded our limited knowledge about their ecological preferences. Both species seem to tolerate the relatively high concentrations of nutrients and silicates, reflecting the eutrophication process in the ecosystems they colonize.

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