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RESEARCH ARTICLE

Albertania and *Egbenema* gen. nov. from Nigeria and the United States, expanding biodiversity in the Oculatellaceae (cyanobacteria)

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Abstract

Knowledge of the tropical terrestrial cyanobacterial flora from the African continent is still limited. Of 31 strains isolated from soil and subaerial samples collected in Lagos State, Nigeria, three were found to be in the Oculatellaceae, including two species in a new genus. Subsequently, isolates from microbial mats in White Sands National Park in New Mexico, United States, and from a rock near the ocean in Puerto Rico, United States, were found to belong to the new genus as well. Cyanobacterial isolates were characterized microscopically, sequenced for the 16S rRNA gene and associated ITS region, and phylogenetically analyzed. *Egbenema* gen. nov., with three new species, as well as two new species of *Albertania* were differentiated from all other Oculatellaceae. Both genera belong to a supported clade within the Oculatellaceae that includes *Trichotorquatus* and *Komarkovaea*. The two new species of *Albertania, A. egbensis* and *A. latericola*, were from the same sample, but were evolutionarily separate based on 16S rRNA gene phylogenies, percent identity below the 98.7% threshold, and ITS rRNA percent dissimilarity >7.0%. *Egbenema aeruginosum* gen. et sp. nov. was phylogenetically separated from *Trichotorquatus* and *Albertania* but was in a clade with other strains belonging to *Egbenema*. The two *Egbenema* strains from the United States are here named *Egbenema epilithicum* sp. nov. and *Egbenema gypsiphilum* sp. nov. Our results support the hypothesis that further species discoveries of novel cyanobacteria will likely be made in soils and subaerial habitats, as these habitats continue to be studied, both in tropical and temperate biomes.

KEYWORDS
16S-23S ITS rRNA, gypsum, ITS rRNA dissimilarity, Lagos Nigeria, Oculatellales, polyphasic approach, Puerto Rico, Synechococcales, terrestrial cyanobacteria, tropical

Abbreviations: BI, Bayesian Inference; CBFS, České Budějovice Faculty of Science; CHAB, Harmful Algae Biology Laboratory in the Institute of Hydrobiology, China; CIPRES, Cyberinfrastructure for Phylogenetic Research; DIC, differential interference contrast; ESS, estimated sample size; GTR+G+1, general time-reversible Markov model with gamma distributed rates mixed with invariable sites; ITS, internal transcribed spacer; LM, light microscope; ML, maximum likelihood; NCBI, National Center for Biotechnology Information; PAUP, phylogenetic analysis using parsimony; PCR, polymerase chain reaction; PKUAC, Peking University Algae Collection; PSRF, potential scale reduction factor; WHSA, White Sands National Park; Z8, Zehnder 8.

INTRODUCTION

There has been considerable revision of the cyanobacterial taxonomic system in the past two decades. More than 180 genera have been described since 2000 (Strunecký et al., 2023). Phylogenetic analysis of molecular data, particularly 16S rRNA gene sequence data, has demonstrated that many genera are polyphyletic when old morphological concepts are applied, for example, *Anabaena*, *Nostoc*, *Tolypothrix*, *Calothrix*, *Oscillatoria*, and *Leptolyngbya* among others (Berrendero-Gomez et al., 2016; Genuário et al., 2015; Hauer et al., 2014; Komárek et al., 2014; Mühlsteinová et al., 2018; Rajaniemi et al., 2005; Zammit et al., 2012). Recently, a number of these genera have been split into multiple, more narrowly defined genera (see review of Strunecký et al., 2023). A careful polyphasic characterization including DNA-based information can correct the evident polyphyly in cyanobacterial genera and can result in the description of new genera or the unification of different generic entities.

In addition to revisionary work, there has been rapid discovery of novel cyanobacterial clades, particularly in tropical regions. For instance, in Brazil, a very active cyanobacterial research group has described a collection of over 20 new genera from the Mata Atlantica and other parts of Brazil (Alvarenga et al., 2016, 2017, 2021; Da Silva Malone et al., 2015; De Lima & Branco, 2020; Fiore et al., 2007; Gama et al., 2019; Genuário et al., 2015, 2018; Hentschke et al., 2016, 2017; Martins et al., 2016; Martins & Branco, 2016; Sant'Anna et al., 2010; Vaz et al., 2015; Werner et al., 2008). Other progress in the description of tropical genera has occurred in India and China, where many Nostocalean taxa have been discovered (Bagchi et al., 2017; Cai et al., 2020; Cai & Li, 2019; Cai, Li, Geng, & Li, 2019; Cai, Li, Yang, et al., 2019; Dadheech et al., 2012; Kumar et al., 2022; Pal et al., 2022; Saraf et al., 2018, 2019). However, the cyanobacterial flora of tropical Africa has been less studied, although *Lagosinema* was recently described from Nigeria (Akagha et al., 2019) from a brackish lagoon, and our knowledge of the terrestrial cyanobacterial flora from Nigeria is even more limited.

Recently, we have been studying the cyanobacteria from dryland soils including those developing biological soil crusts. These studies have led to the discovery of many new species in interesting recent or poorly understood genera, including *Mojavia* (Baldarelli et al., 2022; Řeháková et al., 2007), *Nodosilinea* (Perkerson et al., 2011; Vázquez-Martínez et al., 2018), *Kastovskya* (Mühlsteinová, Johansen, Pietrasiak, & Martin, 2014), *Trichocoleus* (Mühlsteinová, Johansen, Pietrasiak, Martin, Osorio-Santos, & Warren, 2014), *Symplocastrum* (Pietrasiak et al., 2014), *Oculatella* (Becerra-Absalón et al., 2020; Osorio-Santos et al., 2014), *Roholtiella* (Bohunická et al., 2015), *Chroakolemma* (Becerra-Absalón et al., 2018), *Myxacorys* (Pietrasiak et al., 2019), and *Trichotorquatus* (Pietrasiak et al., 2021). When we observed a putative new terrestrial genus in Nigeria, we examined other strains we had in our combined collections that had similar morphologies and sequences and discovered the new genus was also represented in two other isolates, one from microbial mats in New Mexico and one from a subaerial habitat in Puerto Rico. We here describe the new genus and all five species from our culture collections.

METHODS

Sample collection and isolation

Soil and rock samples were collected from the southwestern part of Nigeria in a community northwest of Lagos in Lagos State (6°31′48.9936″ N, 3°16′44.4504″ E) in August 2019 for isolation of cyanobacteria. The area is characterized by a tropical climate with distinct wet and dry seasons and mean annual rainfall of ca. 1700 mm (Ahamefule & Mbagwu, 2007). Microbial mats from ephemerally ground water inundated gypsum sediments were sampled in June 2016 in White Sands National Park, New Mexico, United States (32°52′14.88″ N, 106°17′2.4″ W). Lastly, cyanobacteria were sampled from a rock near the seashore at Punta Viento, Puerto Rico in August 2013 (17°58′14.4″ N, 65°58′31.3″ W) as part of a larger project studying cyanobacteria of that island.

Strains were isolated from natural populations into unicyanobacterial cultures using standard microbiological methods, including enrichment agar plates and direct isolation from the original samples into liquid Z8 medium (Carmichael, 1986; Kotai, 1972). The Nigerian samples were plated at two dilutions (10^{-3} and 10^{-4}) and then incubated at 22°C, illuminated under a 12:12 h light:dark cycle under warm white fluorescent lights and monitored for growth. Microbial mat samples from New Mexico and the Puerto Rican samples were also dilution plated at same concentrations but incubated at 15°C under warm white fluorescent lights with a 16:8 h light:dark cycle. After the growth of cyanobacterial colonies was observed (about 4 weeks), isolated colonies were picked and transferred into test tubes with liquid Z8 media using a dissecting stereomicroscope to obtain unicyanobacterial isolates. When Nigerian and Puerto Rican cultures attained visible biomass in liquid media, they were transferred to agar-solidified Z8 medium in capped test tubes. Cultures from White Sands National Park were kept in liquid Z8 due to repeatedly observed poor growth and health on solid Z8 media.

Morphology and typification

The Nigerian and Puerto Rico strains were characterized microscopically using an Olympus BX60 photomicroscope with Nomarski DIC optics at 1000X and an Olympus SC50 camera system with CellSens software for imaging. Morphological observations of the New Mexico strains were done with a Zeiss Axio-Imager stereoscope. Filament and cell dimensions of each cell type were measured as part of the characterization process throughout the life cycle, at approximately at 1, 2, and 4 months following transfer to fresh media. Thylakoid position was determined in LM by position of the chromoplasm within the cytoplasm. A herbarium voucher was prepared for all strains by immobilizing each culture on glass fiber filters and allowing them to air dry for 3 d. Subsequently, the filters were mounted on herbarium cardstock and placed in herbarium envelopes designed for lichen accessions. The herbarium folder containing the dried herbarium mount was then deposited in the Herbarium at the University of South Bohemia, České Budějovice in the Czech Republic. The reference cultures of each of the strains will be maintained in the algal culture collection facility at John Carroll Algal Culture Collection and the University of Nevada, Las Vegas Culture Collection (the WHSA—WHite SAnds National Park—strains) and will be available upon request. The new taxa described in this paper have been described in accordance with the requirements and recommendations of the International Code of Nomenclature for Algae, Fungi, and Plants (Turland et al., 2018).

Molecular characterization

DNA was extracted from unicyanobacterial cultures using the Qiagen DNeasy PowerSoil® Pro Kit (Venlo, The Netherlands) following the manufacturer's protocol. The extracted DNA was visualized on a 1% agarose gel to ensure that a good extraction was achieved, and then stored at –20°C. A fragment of the 16S rRNA gene and the full 16S–23S ITS rRNA region was amplified by polymerase chain reaction (PCR) using primers VRF-1R and VRF-2F (Flechtner et al., 2002, after Nübel et al., 1997; Wilmotte et al., 1993). The amplification was then run on a C1000 Thermocycler (BIORAD, Hercules, CA, USA) using the following PCR cycle: 94°C for 5 min followed by 35 cycles of 94°C denaturation temperature for 45 s, 57°C annealing temperature for 45 s, and 72°C extension temperature for 135 s. At the end of these cycles, a final extension period at 72°C for 300 s was provided, followed by an indefinite hold at 4°C. The PCR products were visualized on 1% agarose gel and cloned into the pSC-A-amp/kan plasmid of the StrataClone PCR Cloning kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. The clones were isolated using the QIAGEN QIAprep Spin Miniprep kit (Venlo, The Netherlands). The presence of an insert was confirmed by *Eco*RI digestion. For each of the strains, three plasmids were sent for sequencing to Functional Biosciences, Inc. (Madison, Wisconsin, United States). Primers M13 forward and M13 reverse (located in the plasmid DNA) and internal primers 5, 7, and 8 were used (Flechtner et al., 2002). Raw sequences were aligned, error proofed, and assembled to contigs for each clone using the Sequencher Software (Version 4.8; Ann Arbor, MI, United States). The three clones were used to construct consensus sequences where possible. Alignments of multiple strains with other sequences available in GenBank were constructed with ClustalW (Larkin et al., 2007), with the position of indels being manually corrected based upon secondary structure (Řeháková et al., 2014). After alignment, the data were converted to Nexus and Phylip files for phylogenetic analysis.

Phylogenetic analysis

Bayesian inference (BI) and maximum likelihood (ML) analyses were performed using MrBayes on XSEDE 3.2.6 (Ronquist et al., 2012) and RAxML-HPC2 on XSEDE 8.2.10 (Stamatakis, 2014), respectively, both on the CIPRES Science Gateway supercomputing facility (Miller et al., 2015). Analyses were run on an alignment of 427 cyanobacterial sequences (16S rRNA gene, ~1162 nucleotides) belonging to the orders Leptolyngbyales, Oculatellales, Nodosilineales, Prochlorotricales, Synechococcales, Pseudanabaenales, Acaryochloridales, Thermostichales and Gloeobacteriales. For both analyses, the GTR + G + I evolutionary model was used. The Bayesian Inference analysis was run for 80 million generations, discarding the first 25% of samples as burn-in, at which time it achieved an average standard deviation of split frequencies of 0.035. The potential scale reduction factor (PSRF) was ≤1.01 for all parameters, indicating chains converged, and the minimum estimated sample size (ESS) was >270 for all parameters, indicating all parameters were adequately sampled in this analysis. Maximum likelihood analysis was conducted using the same alignment and model that was used for the BI analysis and included 1000 bootstrap iterations. Trees were initially viewed using Fig Tree (Rambaut, 2009). A collapsed Bayesian tree was prepared in Adobe Illustrator (CS5.1 suite, Adobe Systems, San Jose, California) with ML bootstrap values mapped on to nodes. The uncollapsed tree is archived in Figure S1 in the Supporting Information.

Percent similarity of 16S rRNA gene sequences was determined using the SHOWDIST command in PAUP to reveal similarity among strains of interest (Swofford, 1998). Percent dissimilarity of aligned ITS rRNA regions was calculated from *p*-distance determined in PAUP. The hypothetical ITS D1–D1′, Box–B, V2 and V3 helices were identified in the sequence based upon conserved basal clamp regions of each helix and position within the ITS (Figure S1). Secondary structures of these

helices were derived using Mfold (Zuker, 2003) with the default settings, except for draw mode, which was set at Untangle with Loop Fix. These Mfold structures were then re-drawn manually in Adobe Illustrator CS5.1.

RESULTS

Taxonomy

Albertania egbensis M.U. Akagha et J.R. Johansen sp. nov. (Figure 1a–e)

Diagnosis: Morphologically indistinguishable from other *Albertania* species but differs from all other described *Albertania* species by the presence of a cytosine–cytosine mismatch in the lower part of the D1–D1′ helix and a single guanine mismatch in the upper part of the D1–D1′ helix. Also differs from other species in the structure and sequence of the Box-B helix of the ITS.

Description: In culture thallus a dark olive to dark blackish green flat mat, not penetrating the agar. Filaments straight or spiraled, entangled, with rare false branching, with a single trichome per sheath, 2.0–2.6 μm wide (Figure 1a). Sheaths narrow, clear, tightly adherent to the trichomes, at times extending beyond the end cell, obligately present. Trichomes without constrictions at crosswalls, 1.6–2.2 μm wide (mean 2.0 μm). Cells purplish green, with thylakoids parietal along outside walls, without granules or becoming granulated in the centroplasm, isodiametric to longer than wide, 2.0–4.6 μm long (mean 2.8 μm). Apical cells rounded, sometimes more yellowish

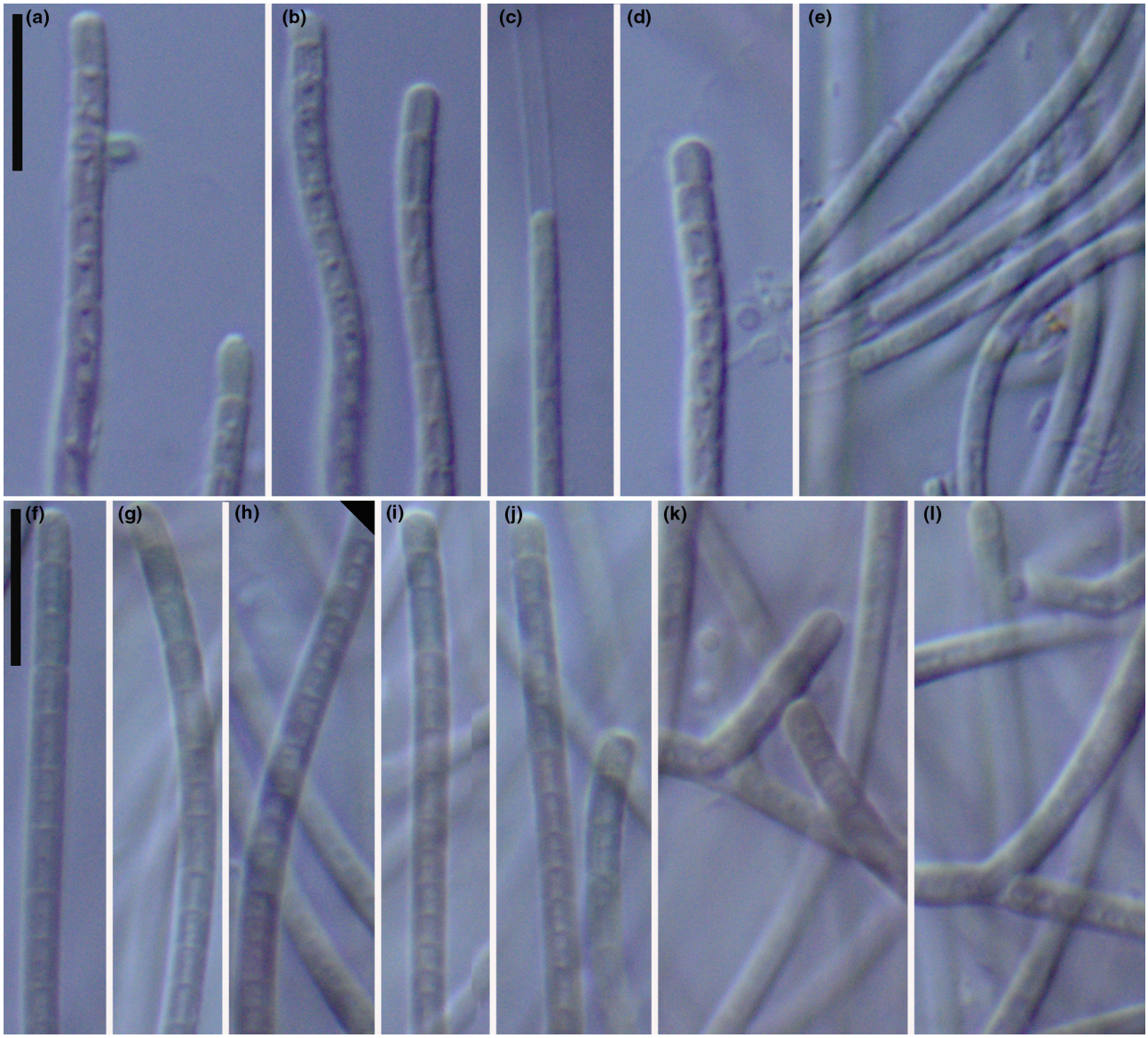

FIGURE 1 Morphological characteristics of *Albertania egbensis* and *A. latericola*. (a–e) *A. egbensis*; (a, b, d) Yellowish apical cells and granules. (c) Elongated sheath. (e) Thinner filaments. (f–j) *A. latericola*; Yellowish apical cells and granules. (k, l) Evident false branching. Scale = 10 μm; applies to all figures.

than vegetative cells within the trichome, 2.0–4.0 μm long (mean 2.8 μm). Hormogonia infrequent, produced in the absence of necridia by simple binary fission.

Holotype here designated: CBFS! A-132-1, Herbarium of the University of South Bohemia. Dried, metabolically inactive material on filter prepared from the reference strain.

Type locality: Brick in yard of a house at 18 Femi Osobu Street, Cele-Egbe, Lagos, Nigeria, (6°31′48.9936″ N, 3°16′44.4504″ E). Sampled by Mildred Akagha on 20 August 2019.

Etymology: Named for *Egbe*, the community in which it was found.

Reference strain: N14-MA1.

Albertania latericola M.U. Akagha et J.R. Johansen sp. nov. (Figure 1f–l)

Diagnosis: Morphologically indistinguishable from other *Albertania* species, but differs from all other described *Albertania* species by the presence of a 5′–AA—A–3′ mismatch in the upper part of the D1–D1′ helix. Also differs from other species in the structure and sequence of the Box-B helix of the ITS.

Description: In-culture thallus a dark olive to dark blackish green flat mat, not penetrating the agar. Filaments straight, entangled, with infrequent false branching, with a single trichome per sheath, 2.2–2.6 μm wide. Sheaths narrow, clear, tightly adherent to the trichomes, at times extending beyond the end cell, obligately present. Trichomes without constrictions at crosswalls, 2.0–2.6 μm wide (mean 2.2 μm). Cells purplish green, with thylakoids parietal along outside walls, without granules or becoming granulated in the centroplasm, 2.2–4.6 μm long (mean 3.2 μm). Apical cells sometimes more yellowish than vegetative cells within the trichome, 2.0–4.0 μm long (mean 2.9 μm). Hormogonia and necridia unobserved.

Holotype here designated: CBFS! A-130-1, Herbarium of the University of South Bohemia. Dried, metabolically inactive material on filter prepared from the reference strain.

Type locality: Brick in yard of a house at 18 Femi Osobu Street, Cele-Egbe, Lagos, Nigeria, (6°31′48.9936″ N, 3°16′44.4504″ E). Sampled by Mildred Akagha on 20 August 2019.

Etymology: L. *later* = brick, *–icola* dwelling upon. The *Albertania* living on brick.

Reference strain: N14-MA3.

Egbenema M.U. Akagha et J.R. Johansen gen. nov

Description: Filaments simple, with infrequent false branching, with sheaths obligately present, less than 3 μm wide. Trichomes slightly constricted at the crosswalls. Cells with parietal thylakoids, longer or shorter than wide.

Etymology: Named for the city from which it was isolated *Egbe*, Nigeria. *-nema* = thread.

Type species: *Egbenema aeruginosum* M.U. Akagha et J.R. Johansen.

Egbenema aeruginosum M.U. Akagha et J.R. Johansen sp. nov. (Figure 2a–f)

Diagnosis: Differs from all other described *Egbenema* species by the presence of four nucleotides in the terminal loop of the D1–D1′ helix as well as other differences in structure in both the D1–D1′ helix and the Box-B helix of the ITS.

Description: In culture thallus a blue-green flat mat, not penetrating the agar. Filaments straight, with a single trichome per sheath, with infrequent false branching, 2.2–2.8 μm wide. Sheaths narrow, clear, tightly adherent to the trichomes, at times extending beyond the end cell, commonly but facultatively present. Trichomes slightly constricted at the crosswalls, 2.0–2.6 μm wide (mean 2.2 μm). Cells bright blue-green, with thylakoids parietal along outside walls but frequently only evident on one side of the trichome, occasionally parietal along crosswalls as well as outside walls, lacking granulation, 1.2–2.8 μm long (mean 1.9 μm). Apical cells slightly longer than interior cells when mature, 1.8–3.2 μm long (mean 2.2 μm). Hormogonia and necridia unobserved.

Holotype here designated: CBFS! A-131–1, Herbarium of the University of South Bohemia. Dried, metabolically inactive material on filter prepared from the reference strain.

Type locality: Soil in the yard of a house at 18 Femi Osobu Street, Cele-Egbe, Lagos, Nigeria, (6°31′48.9936″ N, 3°16′44.4504″ E). Sampled by Mildred Akagha on 20 August 2019.

Etymology: L. *aeruginosus* = bright blue-green.

Reference strain: N15-MA6.

Egbenema epilithicum J.R. Johansen et M.U. Akagha sp. nov. (Figure 2g–k)

Diagnosis: Differs from other *Egbenema* species in the frequent presence of false branching and entangled trichomes. Also differs from other *Egbenema* species by the presence of an additional bilateral bulge (5′–AUG—GA–3′) below the subterminal bilateral bulge of the D1–D1′ helix as well as other differences in structure in both the D1–D1′ helix and the Box-B helix of the ITS.

Description: In culture, thallus a bright blue-green flat mat, not penetrating the agar. Filaments straight and coiled, at times irregularly entangled or coiled

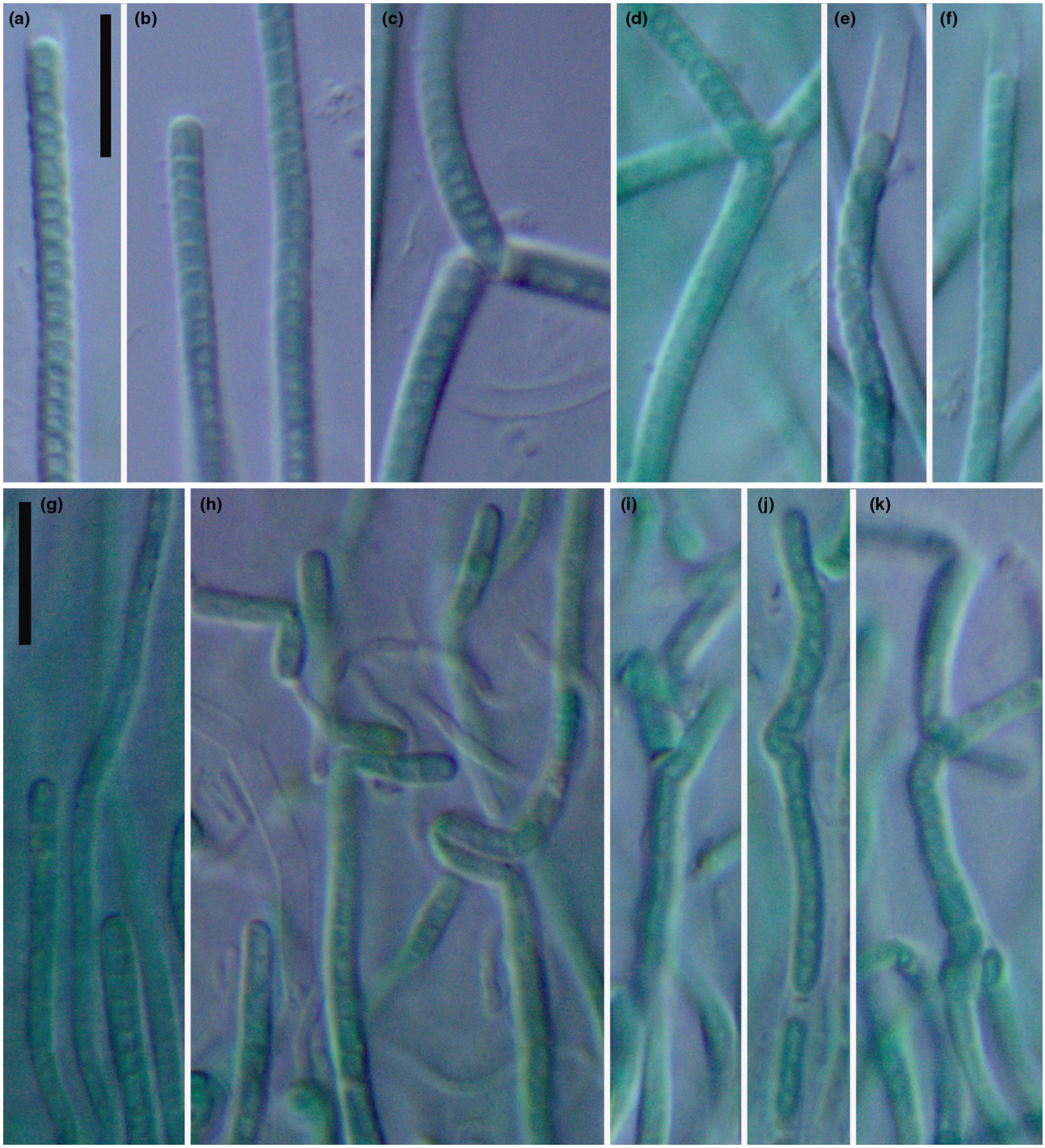

FIGURE 2 Morphological characteristics of *Egbenema aeruginosum* and *E. epilithicum*. (a–f) *E. aeruginosum*; (a–c) Apical cells slightly longer than interior cells when mature. (d) False branching. (e, f) Sheaths extending beyond the end cell. (g–j) *E. epilithicum*. (g) Apical cells longer or shorter than inner cells. (h–k) Formation of false branching. (j) Hormogonia formed by simple fragmentation. Scale = 10 μm; applies to all figures.

within the sheath, with a single trichome per filament, free or with sheath, with frequent false branching, 1.8–3.0 μm wide. Sheaths narrow, clear, tightly adherent to the trichomes, at times extending beyond the end cell, facultatively present. Trichomes usually without constrictions at crosswalls, sometimes clearly constricted, 1.6–3.0 μm wide (mean 2.2 μm). Cells blue-green, with thylakoids parietal along outside walls, without granules, 1.6–4.0 μm long (mean 2.4 μm). Apical cells longer or shorter than inner cells 2.0–4.0 μm long (mean 2.8 μm). Necridia unobserved. Hormogonia formed by simple fragmentation, 2 to several cells in length.

Holotype here designated: CBFS! A-129-1, Herbarium of the University of South Bohemia. Dried,

metabolically inactive material on filter prepared from the reference strain.

Type locality: Dark algal film growing on rock near the ocean at Punta Viento, Puerto Rico, USA. Sampled August 9, 2013. (17°58′14.4″ N, 65°58′31.3″ W). Sampled by J.R. Johansen, J. Kaštovský, J. Mareš, and M. Bohunická on 9 August 2013.

Etymology: L. *epilithicus* = growing on rock.

Reference strain: CT225.

Egbenema gypsiphilum Pietrasiak, J.R. Johansen et M.U. Akagha sp. nov. (Figure 3a–f)

Diagnosis: Differs from other species of *Egbenema* in the presence of yellowish apical cells. Also differs from other *Egbenema* species by the presence of an enlarged terminal loop of 16 nucleotides in the D1–D1′ helix, a large bilateral bulge in mid-helix of the Box-B helix, a shorter Box-B helix with longer spacer region, as well as other differences in structure in both the D1–D1′ helix and the Box-B helix of the ITS.

Description: In liquid culture, thallus a bright blue-green floating mat. Filaments straight and coiled, free or with sheaths, with frequent false branching, 2.2–2.8 μm wide. Sheaths narrow, clear, tightly adherent to the trichomes, at times open and extending beyond the end cell, facultatively present. Trichomes mostly constricted at crosswalls, appearing unconstricted when enclosed in a sheath, 2.0–2.6 μm wide (mean 2.1 μm). Cells blue-green, with thylakoids parietal along outside walls, often with a single large granule in the centroplasm, sometimes with reddish inclusions toward the apices, 1.8–3.6 μm long (mean 2.7 μm). Apical cells longer or shorter than inner cells, frequently more yellowish in color, 2.0–4.2 μm long (mean 3.1 μm). Necridia present but rare. Hormogonia formed by simple fragmentation.

Holotype here designated: CBFS A183-1! Herbarium of the University of South Bohemia, Czech Republic. Dried, metabolically inactive material on filter prepared from the reference strain.

Type locality: microbial mat in ground water inundated gypsum sediment, White Sands National Park, New Mexico, United States (32°52′14.88″ N, 106°17′2.4″ W), elevation 1,206 m. Sampled by Nicole Pietrasiak, Radka Hauerová and Megan Stovall on June 8, 2016.

Etymology: L. *gypsiphilum* = gypsum loving.

Reference strain: WHSA1-4-NP1A.

Molecular analysis

Phylogeny

The families Oculatellaceae, Leptolynbyaceae, Trichocoleusaceae, and Pseudanabaenaceae are as they were defined originally (Mai et al., 2018), and they agree

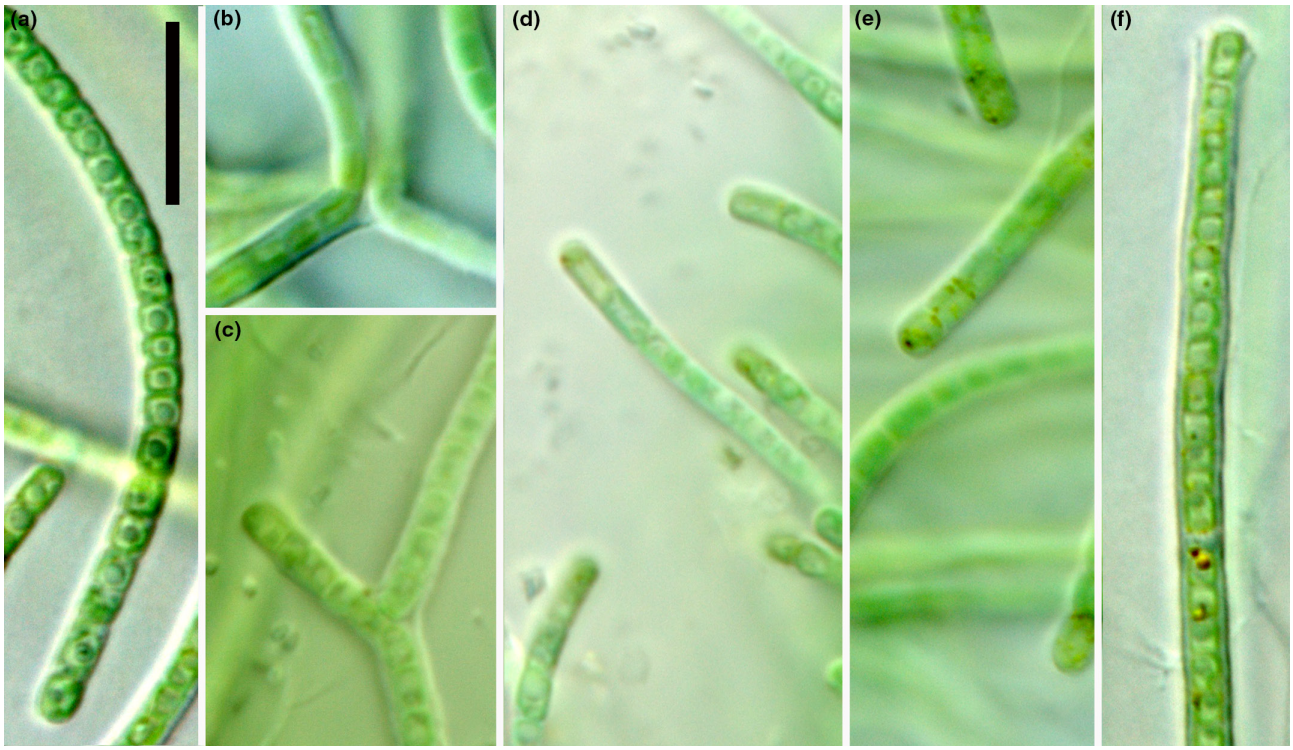

FIGURE 3 Morphological characteristics of *Egbenema gypsiphilum* strain WHSA1-4-NP1A. (a) Large granules in the centroplasm. (b, c) False branching. (d) Yellowish apical cells. (d, e) Dark reddish inclusions towards the apices. (f) Thickened firm sheath. Scale = 10 μm; applies to all photos in the figure.

with what was found subsequent to that work (Becerra-Absalón et al., 2018; Pietrasiak et al., 2019, 2021). The Prochlorotrichaceae (as defined in Mai et al., 2018) is separated into two clades (*Procholothrix* and relatives and *Nodosilinea* and relatives; Figure 4). Revision of this group has already resulted in the recognition of two different families, the Nodosilineaceae and Prochlorotrichaceae (Strunecký et al., 2023), which agrees with our current observations. The family Oculatellaceae had fair support in the Bayesian Inference tree (0.88; Figure 4), but some of the taxa within the family are unresolved in a polytomy that includes *Oculatella*, *Timaviella*, *Tildeniella*, and a number of genera in the clade that includes our taxa of interest (Figure 4). The node containing *Albertania*, *Komarkovaea*, *Egbenema*, and *Trichotorquatus* has fair support in the BI analysis (0.86 posterior probability), but the relationship of these genera to each other is unresolved. Our strains fall into two genera: *Egbenema* and *Albertania*. From this analysis, the two *Albertania* strains, both from the same soil sample, fall into different clades and consequently represent two separate species of *Albertania* (*A. egbensis* and *A. latericola*). *Egbenema* has three major clades (*E. epilithicum*, PKUAC strains, and the *E. aeruginosum*/*gypsiphilum* group) and all fall in the same clade with a strongly supported node (1.0/93). The strains in the *E. aeruginosum*/*gypsyphila* clade appear to be separate species, but we only describe those strains for which we have seen material (N15-MA6 and WHSA1-4-NP1A/WHSA1-1-NP3C). We anticipate that some of the other strains (e.g., PKUAC strains) will be described by others who have possession of the strains.

16S rRNA gene percent similarity

Percent 16S rRNA gene similarity of strains within *Albertania* and *Egbenema* showed high similarity among strains (Table 1). Similarity values <94.5% are considered strong evidence that those strain pairs belong to different genera, while similarity values <98.7% are considered strong evidence that those strain pairs belong to different species (Yarza et al., 2014). Values above these thresholds are considered uninformative. All the *Albertania* species (*A. egbensis*, *A. latericola*, *A. skiophila*, *A. alaskaensis*, and *Albertania* sp. VRUC184) are below the 98.7% threshold (Table 1), which indicates that they are different species of *Albertania*. Likewise, all of the *Egbenema* species are below the 98.7% similarity threshold and are supported as separate species. The sequences of PKUAC strains within the *Egbenema* clade are all above the threshold and likely represent a single species of *Egbenema*. Members of the genus *Albertania* lie slightly above or below the 94.5% threshold (Table 1), but given their phylogenetic separation (Figure 4), it is reasonable to recognize *Egbenema* and *Albertania* as separate genera. *Egbenema* is more closely related to *Trichotoquartus* in the ML phylogeny than it is to *Albertania* (Table 1), although based on percent similarity, *Trichotorquatus* is clearly separated from both *Albertania* and *Egbenema*.

ITS rRNA percent dissimilarity

The percent dissimilarity between species within the genera *Albertania* and *Egbenema* shows that all species within these genera are different in that they have a value between 10% and 34%. Values >7.0% dissimilar are considered strong evidence that the pair of strains in the comparison are different species (Erwin & Thacker, 2008; Osorio-Santos et al., 2014; Pietrasiak et al., 2019, 2021). *Egbenema aeruginosum* is 15% dissimilar to *E. gypsiphilum* and 23% different from *E. epilithicum*. *Albertania latericola* and *A. egbensis* are also clearly different from each other with a percent dissimilarity of 7.9%. Interspecific comparisons of all described *Albertania* species are above the 7.0% dissimilarity threshold and are supported as separate species by this criterion (Table 2).

ITS structures

While all the D1–D1′ helices in *Albertania* are similar in their basal regions up through the helix between the C–U mismatch and the second mismatch in the helix, differences are evident above that point (Figure 5). *Albertania skiophila* and *A. alaskaensis* are distinctly different in the D1–D1′ helix structure from each other and from the Nigerian species (Figure 5a–d), and our study confirms these species based on secondary structure analysis. *Albertania latericola* and *A. egbensis* differ from each other and from the previously described species in the size of the subterminal bilateral bulge and in the sequence of the helix below that bulge and in the terminal loop (Figure 5c,d). All of the D1–D1′ helices in the *Egbenema* species were markedly different in both sequence and structure above the basal unilateral bulge. Four strains, *E. aeruginosum* N15-MA6, *Egbenema* sp. PKUAC-A174, *Egbenema* sp. CY40, and *E. gypsiphilum* WHSA1-4-NP1A shared an unmatched cytosine residue opposite the basal 3′ unilateral bulge, while the other two species had an additional base pairing either before or after the bilateral bulge (Figure 5f,h). *Egbenema aeruginosum* had the tightest helix with fewest mismatched pairs in the main central helix (Figure 5e). The terminal loop was enlarged in *Egbenema* strains CY40 and WHSA1-4-NP1A (Figure 5i,j) but reduced in CHAB TP201703 (Figure 5h).

The Box-B helices in species of *Albertania* (*A. skiophila*, *A. alaskaensis*, *A. latericola*, and *A. egbensis*) were similar to those in *Egbenema* (*E. aeruginosum*, *E. epilithicum*, *Egbenema* sp. PKUAC-A174, *Egbenema*

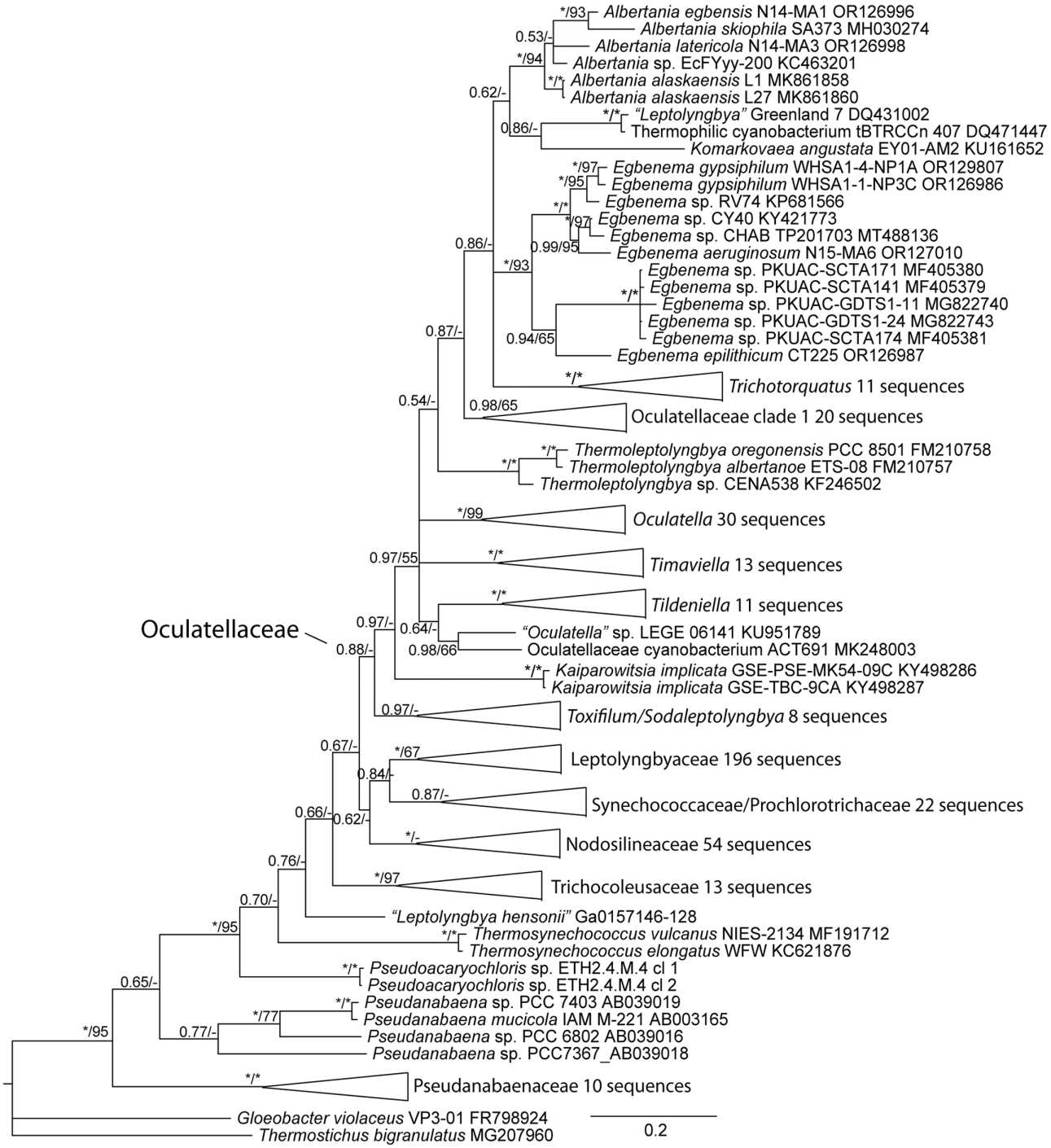

FIGURE 4 Bayesian Inference phylogeny based on 427 partial sequences (nucleotides 343 to end) of the 16S rRNA gene sequence showing lineage relationships within the family Oculatellaceae, with outgroups Leptolyngbyaceae, Prochlorotrichaceae, Trichocoleusaceae, Nodosilineaceae, and Pseudanabaenaceae. Support values at the nodes indicate posterior probability/bootstrap values (BI/ML). Nodes represented by an (*) indicate full support, bootstrap support value of less than 0.50/50% is indicated by (–). Taxa with quotation marks are ones we consider to be incorrect or in need of taxonomic revision.

CY40) in the basal part of the helix, length, and sequence except in the terminal loop (Figure 6a–g,i). However, *Egbenema* sp. CHAB TP201703.3 and *E. gypsiphilum* WHSA1-4-NP1A distinctly differed from all the other species by having shorter sequences (Figure 6h,j). All Box-B helices seen in *Albertania* and *Egbenema* were evidently different from each other. The V3 helices for *A. skiophila*, *A. latericola*, and *A. egbensis* were similar in the basal part, length, and terminal loop (Figure 6k–m). *Egbenema aeruginosum* was different from *E. epilithicum* and *Egbenema* sp. PKUAC-A174 in the terminal loop and basal part by one nucleotide substitution but had similar length and structure (Figure 6n–p). *Egbenema* sp. CHAB TP201703.3 was distinctly

TABLE 1 Percent similarity of strains related to *Albertania* and *Egbenema* based on a partial 16S rRNA gene sequence ~1160 nt. long.

	A. alaskaensis L27	*Albertania* sp. EcFYyy 200	*A. Skiophila* SA373	*Albertania* sp. VRUC206	*Albertania* sp. VRUC201	*Albertania* sp. UIC 10061	*A. Egbensis* N14 MA1	*Albertania* sp. VRUC184	*A. Latericola* N14 MA3
Albertania sp. EcFYyy 200	98.8								
A. skiophila SA373	96.6	97.2							
Albertania sp. VRUC206	97.3	97.1	98.2						
Albertania sp. VRUC201	97.4	97.4	98.5	99.5					
Albertania sp. UIC 10061	97.7	97.7	98.3	98.5	98.8				
A. egbensis N14 MA1	97.9	97.9	98.1	98.5	98.6	99.0			
Albertania sp. VRUC184	96.9	97.5	98.6	98.2	98.5	98.3	98.5		
A. latericola N14 MA3	97.8	98.2	96.9	97.8	98.0	98.4	97.7	97.2	
Egbenema sp. CHAB TP201703.3	95.4	95.0	94.5	95.4	95.3	95.7	95.8	95.0	94.4
Egbenema sp. CY40	95.3	95.7	94.5	95.0	94.9	95.3	95.8	94.6	94.7
E. aeruginosum N15-MA6	95.2	95.2	94.5	95.0	94.9	95.5	95.9	94.6	94.6
Egbenema sp. RV74	95.1	95.2	94.0	94.5	94.4	95.0	95.3	94.3	94.2
E. epilithicum CT225	95.6	95.6	94.5	95.0	95.1	95.9	95.4	94.6	95.4
E. gypsiphilum WHSA1-4-NP1A	95.6	95.7	94.5	94.8	94.7	95.3	95.8	94.7	94.6
Egbenema sp. PKUAC SCTA141	95.4	95.6	94.4	95.4	95.3	95.7	95.7	94.8	94.7
"*Leptolyngbya*" sp. Greenland 7	95.3	95.4	94.3	95.2	95.1	95.6	95.3	94.6	95.1
Komarkovaea angustata EY01-AM2	94.3	94.6	92.5	92.5	92.4	92.9	93.8	92.5	93.7
Trichotorqatus maritimus SMER-A	93.9	93.6	92.9	93.4	93.3	93.8	94.0	93.2	93.3

Note: Similarity <94.5% is considered strong evidence that those strain pairs belong to different genera (Yarza et al., 2014). Similarity <98.7% is considered strong evidence that those strain pairs belong to different species (Yarza et al., 2014). Values above these thresholds are considered uninformative.

longer in the V3 helix structure than all other species (Figure 6q). There was no difference in sequence or structure either in the V3 helix structure of *Egbenema* CY40 and *E. gypsiphilum* WHSA1-4-NP1A (Figure 6r,s) or in the V3 helix structures of *A. skiophila* and *A. egbensis* (Figure 6k,l).

Size of conserved domains of ITS rRNA region

The sizes of the conserved domains for available ITS rDNA region sequences show significant differences, especially in the V2 region between the tRNA genes and the spacers associated with the Box-B (Table 3). The nucleotide regions were grouped into 12 sections based on conserved secondary structures. The Nigerian *Albertania* species had domain lengths that were alike except in the spacer+Box-B+spacer, D4, and V3 helix. *Albertania skiophila* and *A. alaskaensis* differed in all sections except in the tRNA region. *Egbenema aeruginosum* also differed in some of the sections with *E. epilithicum* CT225, *Egbenema* sp. PKUAC-A174, *Egbenema* CY40, *Egbenema gypsiphilum* WHSA1-4-NP1A, and *Egbenema* sp. CHAB TP201703.3 but had similar domain length in

Egbenema sp. CHAB TP201703.3	*Egbenema* sp. CY40	*E. aeruginosum* N15-MA6	*Egbenema* sp. RV74	*E. Epilithicum* CT225	*E. Gypsiphilum* WHSA1-4-NP1A	*Egbenema* sp. PKUAC SCTA141	"*Leptolyngbya*" sp. Greenland 7	*Komarkovaea angustata* EY01-AM2
99.5								
98.2	98.5							
98.1	98.4	97.6						
95.4	95.7	95.4	95.4					
97.9	98.5	98.4	99.2	95.6				
96.1	96.4	95.7	96.4	96.7	96.2			
94.6	95.0	94.5	95.2	94.2	95.3	95.0		
92.2	92.5	92.2	92.7	92.6	92.9	92.9	93.5	
93.1	93.1	93.6	92.7	93.1	93.1	92.3	92.3	91.6

the tRNA and Box-A region. All *Egbenema* species had long V2 regions except for *E. aeruginosum* and *Egbenema* sp. CY40. The domain lengths show supporting evidence that the species boundaries recognized in this paper are valid.

Morphological analysis

Albertania egbensis (Figure 1a–e) has filaments very similar in diameter to the other three species with an almost completely overlapping size range. It lacks the terminal hair reported for *A. alaskaensis*. It is separated from both *A. alaskaensis* and *A. skiophila* by the presence of apical cells that are more yellowish than vegetative cells (Figure 1a–c). *Albertania laterícola* resembles *A. egbensis* in that it also has apical cells that are yellowish in comparison to vegetative cells (Figure 1f,g,i,j), and its size range almost completely overlaps. All *Albertania* species are capable of false branching (Figure 1k,l). These species would be nearly impossible to separate based on morphology alone, and we consider them to be fully cryptic species.

TABLE 2 Percent dissimilarity based on aligned 16S–23S ITS rRNA regions (maximally 570 nucleotides) between species within the genera *Albertania* and *Egbenema*.

***Egbenema* spp.**	***E. aeruginosum* N14-MA1**	***E. Epilithicum* CT225**	***Egbenema* sp. CY40**	***Egbenema* sp. TP201703**	***Egbenema* sp. A174**	***E. Gypsiphilum* WHSA1-4-NP1A**
E. aeruginosum N15-MA6						
E. epilithicum CT225	23.1					
Egbenema sp. CY40	11.0	23.4				
Egbenema sp. CHAB TP201703	16.5	32.4	16.2			
Egbenema sp. PKUAC A174	22.1	31.2	21.7	29.1		
E. gypsiphilum WHSA1-4-NP1A	14.8	34.6	10.4	25.7	32.2	
***Albertania* spp.**	***A. egbensis* N14-MA1**	***A. latericola* N14-MA3**	***A. skiophila* SA373**	***A. alaskaensis* L11**		
Albertania egbensis N14-MA1						
Albertania latericola N14-MA3	7.9					
Albertania skiophila SA373	14.1	12.5				
Albertania alaskaensis L1	17.8	16.9	21.7			

Note: Values >7.0% dissimilar are considered strong evidence that the pair of strains in the comparison are different species.

Egbenema aeruginosum has straight trichomes (Figure 2a–f) as compared with the flexuous trichomes found in both *E. epilithicum* (Figure 2g–k) and *E. gypsiphilum* (Figure 3a–f). *Egbenema aeruginosum* and *E. gypsiphilum* have identical width ranges but differ in the lengths of vegetative cells and apical cells. *Egbenema epilithicum* has slightly narrower trichomes than the other two species. All three species of *Egbenema* are, like *Albertania*, cryptic, and require molecular analysis to determine their identity.

Egbenema and *Albertania* form a clade with two sister taxa, *Komarkovaea* and *Trichotorquatus*. These genera are all fairly cryptic. *Trichotorquatus* is distinguished by a characteristic sheath that forms flared collars around trichomes (Pietrasiak et al., 2021). *Komarkovaeae* produces necridia (Mai et al., 2018), while *Egbenema* and *Albertania* do not, although *Albertania* is still capable of producing hormogonia by simple fission. Dimensions of all four genera have significant overlap.

DISCUSSION

The expanding Oculatellaceae

The family Oculatellaceae has recently been studied in greater detail and a number of new genera and species have been described in the process. Most of these newly described genera and species were collected from terrestrial or subaerial habitats. The type genus of the family, *Oculatella*, is mostly a soil genus (Becerra-Absalón et al., 2020; Jung et al., 2020; Mikhailyuk et al., 2022; Osorio-Santos et al., 2014; Vinogradova et al., 2017). Other taxa were found in subaerial habitats such as caves (Chakraborty et al., 2021; Zammit et al., 2012) or wet walls or hard man-made substrates such as clay pots (Brito et al., 2022; Mai et al., 2018). A few taxa have been described from aquatic environments (Jahodářová, Dvořák, Hašler, Holušová, & Poulíčková, 2017; (Jahodářová, Dvořák, Hašler, & Poulíckova, 2017; Osorio-Santos et al., 2014; Strunecký et al., 2020).

Subaerial habitats, caves, and hypogea have been a great source of Oculatellaceae. For example, *Oculatella subterranea* (Zammit et al., 2012), *Oculatella kauaiensis* (Osorio-Santos et al., 2014), *Albertania skiophila* (Zammit, 2018), *Timaviella circinate*, and *Timaviella karstica* (Sciuto et al., 2017) were all found in cave entrances or hypogea with artificial lighting. Wet walls and frequently wetted hard surfaces represent subaerial habitats, which also have many species, such as *Oculatella cataractarum* (Osorio-Santos et al., 2014), *Cartusia fontana*, *Drouetiella fasciculata*, *D. hepatica*, *D. lurida*, *Kaiparowitsia implicata*, *Komarkovaea angustata*, *Pegethrix bostrychoides*, *P. convoluta*, *P. indistincta*, *P. olivacea*, *Timaviella obliquedivisa*, *Tim. radians*, *Tildeniella*

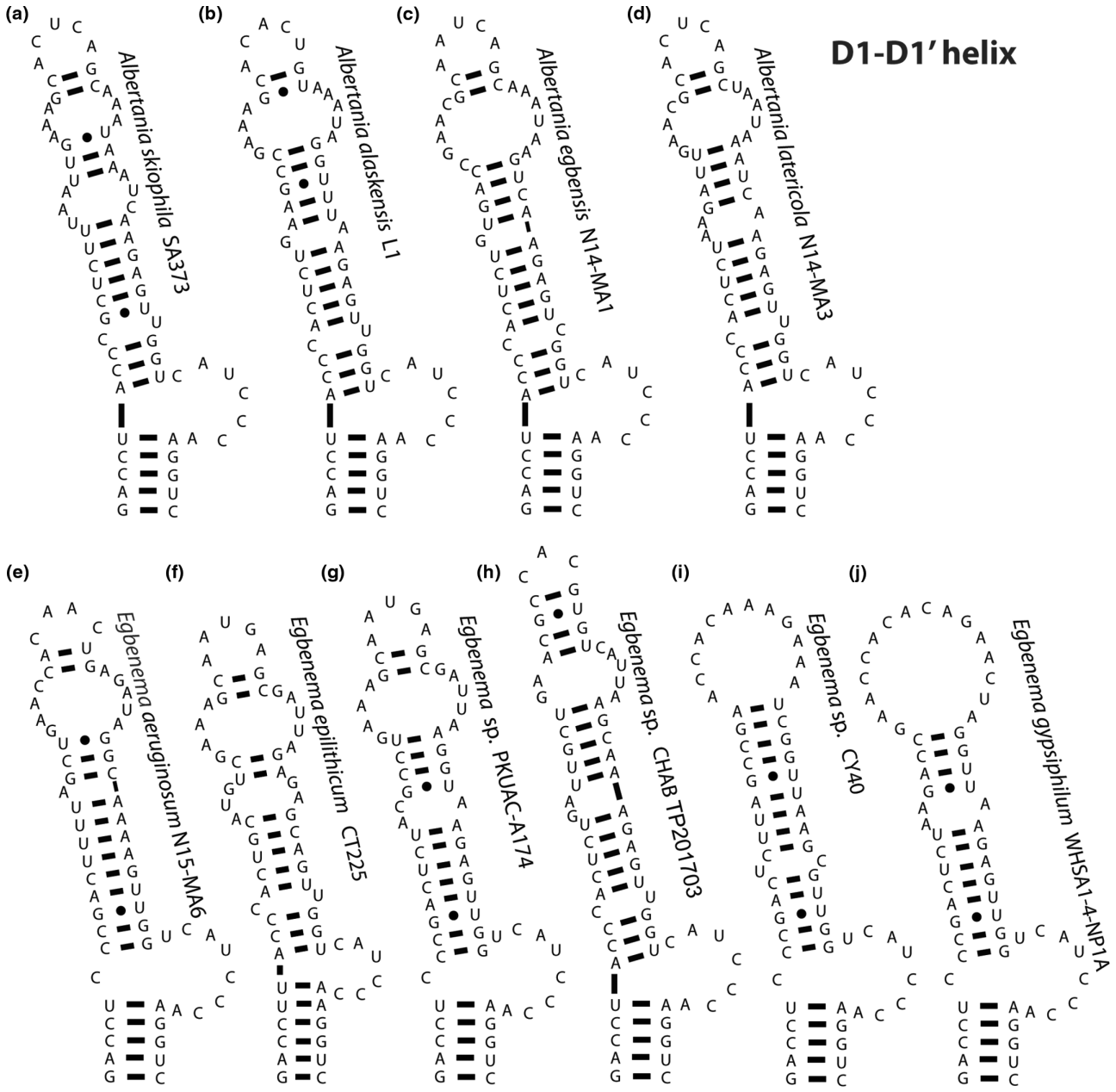

FIGURE 5 Secondary structure of the D1–D1′ helix of previously described species as well as new species of *Albertania*, and newly described genera *Egbenema* for which 16S–23S ITS rRNA sequence data are available. Both new species of *Albertania* are different from already described species with the presence of a cytosine-cytosine mismatch in the lower part of the D1–D1′ helix for *A. egbensis*, and the three-nucleotide mismatch (5′–AA—A–3′) below the subterminal bilateral bulge of the D1–D1′ helix for *A. latericola*. The new genera of *Egbenema* differ from each other in multiple nucleotide positions throughout the helix.

nuda, Til. torsiva, (Mai et al., 2018), and *Oculatella lusitanica* (Brito et al., 2022).

Soils are similar to but harsher than subaerial habitats, yet they support numerous species in the family Oculatellaceae, including, in the genus *Oculatella*, *O. atacamensis*, *O. coburnii*, *O. mojaviensis*, and *O. neakameniensis* (Osorio-Santos et al., 2014); *O. ucrainica* and *O. kazantipica* (Vinogradova et al., 2017); *O. dilatativagina* and *O. leona* (Becerra-Absalón et al., 2020); and *O. crustae-formantes* (Jung et al., 2020). *Aerofilum fasciculatum* (Chakraborty et al., 2021) was recovered from soils in India, while four species in the genus *Trichotorquatus* have been from dryland or desert soils (Pietrasiak et al., 2021), and the recently described *Timaviella dunensis* was described from sand dunes near the Baltic Sea (Mikhailyuk et al., 2022).

The aquatic Oculatellaceae are underrepresented but include *Shackletoniella antarctica* from an Antarctic Lake, both *Albertania alaskaensis* and *Tildeniella alaskaensis* from an Arctic melt-water brook (Strunecký et al., 2020), *Elainella saxicola* from a tropical

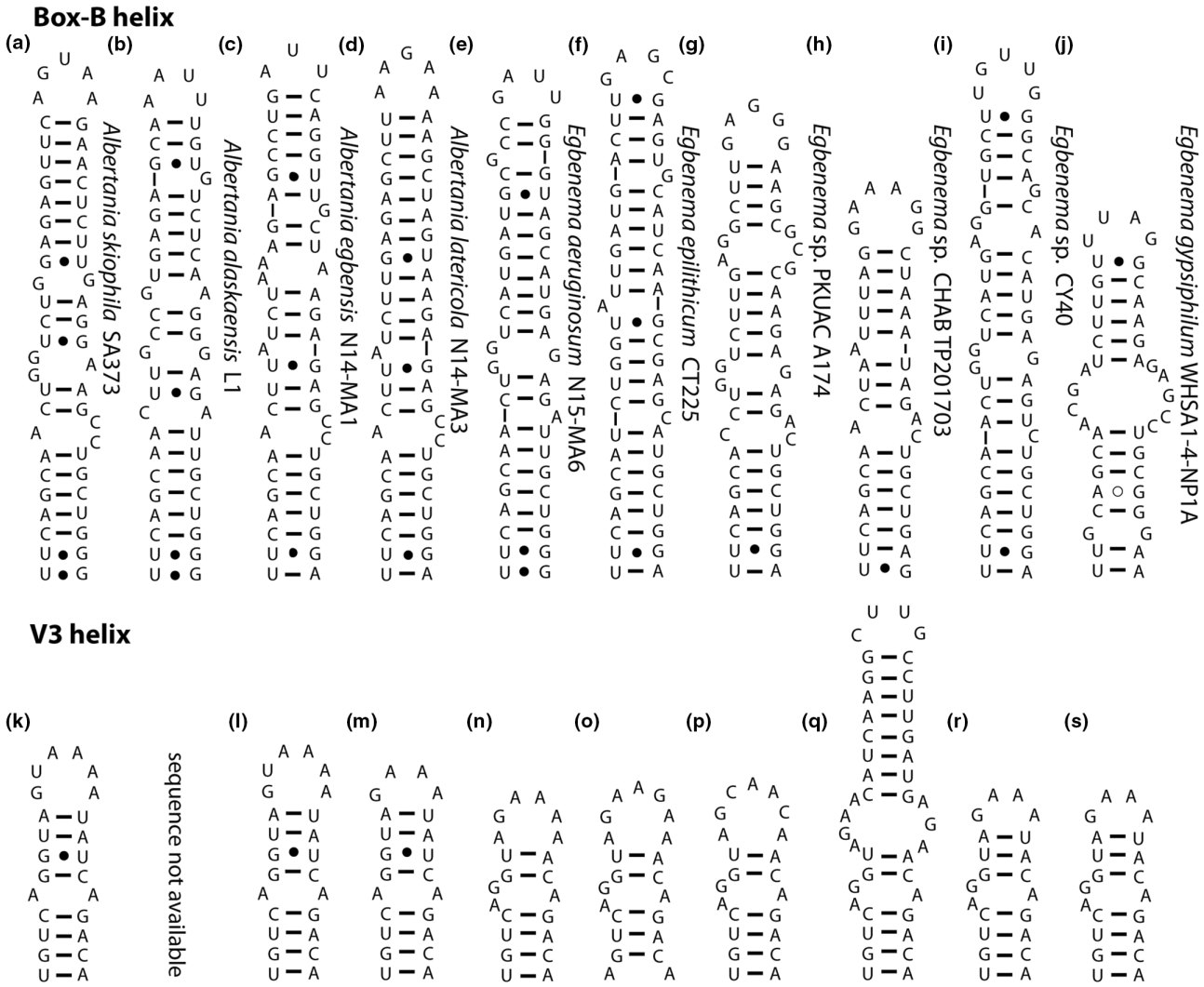

FIGURE 6 Secondary structure of the Box-B (a–j) and V3 (k–s) helices of previously described species as well as new species of *Albertania* and species of *Egbenema* for which 16S–23S ITS rRNA sequence data are available. Strain labels for the Box-B apply to the V3 helices directly below each Box-B structure. The species of *Albertania* and *Egbenema* have different sequence and structure in all Box-B helices. The V3 helix differs in sequence or structure in most cases, although *A. skiophila* and *A. egbensis* have identical V3 helices; *Egbenema* sp. CY40 and *E. gypsiphilum* likewise have identical V3 helices.

vernal pool (Jahodářová, Dvořák, Hašler, Holušová, & Poulíčková, 2017), and *Oculatella hafnerensis* from Hafnersee, Austria (Osorio-Santos et al., 2014). *Thermoleptolyngbya albertanoae*, *T. oregonensis*, *T. sichuanensis*, and *T. hindakiae* from thermal springs are representatives of this distinctive aquatic habitat (Jasser et al., 2022; Sciuto & Moro, 2016; Tang et al., 2021). One genus has been reported from marine habitats, *Calenema singularis* (Brito et al., 2017). We conclude that based on the present evidence, the Oculatellaceae are most widely distributed and most diverse in habitats that are dry for much of the year, with only a few capable of competing in perennially wet habitats. However, the presence of members of this family in a wide range of habitats may indicate that the apparent terrestrial preference is only due to sampling efforts in those habitats. The taxa described in this manuscript are all from soils or subaerial surfaces.

The newly described genus *Egbenema* is quite diverse and broadly distributed. Nigeria is host to the generitype, *E. aeruginosum*, isolated from soil in Egbe, which has a tropical forest climate. *Egbenema epilithicum* was isolated from Puerto Rico, considered to have a tropical monsoonal climate, while *E. gypsiphilum* was isolated from a microbial mat formed on ephemerally groundwater inundated gypsum sediment in the Chihuahuan Desert of New Mexico. Two unnamed species were isolated in China, strain CHAB TP201703 and the PKUAC set of strains. We expect that researchers in China will describe these species in the near future. Strain CY40 from Lonar Lake in India and Strain RV74 from an unspecified habitat in Russia, also belong to this genus.

Albertania, which has two previously described species, is in a clade sister to *Komarkovaea* and several unnamed strains. However, the *Albertania*/

TABLE 3 Nucleotide lengths of conserved domains of the 16S–23S ITS rRNA region.

	Leader	D1–D1′ helix	Spacer + D2	Spacer + D3 + spacer	tRNA-Ile	V2 region	tRNA-ala	Spacer + box-B + spacer	Box-A	D4 region	V3 helix	End sequence	Total length
Albertania alaskaensis L11	8	65	30	17	74	59	73	~106	N/A	N/A	N/A	N/A	N/A
Albertania skiophila SA373	7	64	32	18	74	92	73	113	11	18	24	13	539
Albertania egbensis N14 MA1	8	64	31	17	74	11	73	104	11	17	24	13	447
Albertania latericola N14 MA3	8	64	31	17	74	11	73	106	11	17	22	13	449
Egbenema aeruginosum N15-MA6	7	61	31	17	74	16	73	102	11	17	21	16	446
Egbenema epilithicum CT225	7	63	31	17	74	140	73	95	11	17	31	10	569
Egbenema gypsiphilum WHSA1-4-NP1A	8	62	31	17	74	84	73	164	11	16	21	17	578
Egbenema sp. PKUAC-A174	8	64	31	16	74	136	73	114	11	17	32	2	578
Egbenema sp. CHAB TP201703.3	8	65	30	16	74	119	73	101	11	18	42	15	572
Egbenema sp. CY40	7	61	31	17	74	13	73	100	11	16	21	15	439

Note: *Albertania alaskaensis* was missing the end part of the ITS. A color-coded alignment showing these domains is shown in Table S1 in the Supporting Information.

Komarkovaea clade is in a polytomy with both *Egbenema* and *Trichotorquatus* (Figure 4). We now have two newly described species of *Albertania*, which were collected in close proximity to each other and are morphologically nearly identical. However, they were distinct molecularly, based on phylogeny (Figure 4), low 16S rRNA gene similarity (97.7%, below the 98.7% species threshold, see Table 1), high 16S–23S ITS rRNA region percent dissimilarity (above the 7% species threshold, see Table 2), and the structure of all three conserved helices in the ITS rRNA region (Figures 5 and 6). Every molecular criterion commonly accepted for distinguishing species was in agreement, and consequently, we recognize these two species despite the fact that they are morphologically cryptic. There were six strains within the *Albertania* clade for which we did not have material, and we are hopeful that the holders of these strains will complete the taxonomic work on these unspeciated strains. A perennial problem in cyanobacterial taxonomy is that the generation of sequences for isolates proceeds at a much more rapid pace than the taxonomy, resulting in many "dark taxa" in the sequence databases (Page, 2016). Efforts to fully characterize these dark taxa and bring them into the "light" would advance our understanding of phylogeography and, so, would be highly valuable. Some progress is now being made with completing taxonomy in some genera (e.g., *Oculatella*), but much work remains to be done.

Molecular definition of cyanobacterial taxa

Almost all recent cyanobacterial genera and species have been identified using a polyphasic approach that at the very least, includes both morphological and molecular character sets and frequently utilizes habitat preference as a third criterion. Enough cryptic species and cryptic genera have, at this point, been published that it may be time to reevaluate the criteria for genera and species recognition. Morphology in the thinnest simple filamentous forms is frequently character poor. In the newest system of higher level taxonomy (Strunecký et al., 2023), the Synechococcales sensu Komárek et al. (2014) was fragmented into five filamentous orders (Leptolyngbyales, Oculatellales, Nodosilineales, Prochlorotrichales, and Pseudanabaenales) and five coccoid orders (Synechococcales, Acaryochloridales, Gloeomargaritales, Thermostichales, and Aegeococcales). Morphology alone cannot distinguish any of these orders. They are identified phylogenetically using extensive (genomic) alignments of numerous concatenated protein-coding genes. This approach provides an evolutionarily consistent taxonomic hierarchy for these cyanobacteria, and because of the strength of whole genome phylogenies, this system will likely persist well into the future. However, many genera and

species in what was once the Synechococcales have no sequence data or have only limited data (16S rRNA gene and 16S–23S ITS rRNA region sequences). We anticipate that genera and species will continue to be described utilizing ribosomal sequences, but genomes will become increasingly important for establishing higher level taxonomy, which will undoubtedly include even more orders and families than are currently recognized.

This work follows a pattern established in other studies in the use of several lines of evidence for establishing taxa worthy of recognition. The microbiological thresholds, utilizing 16S rRNA gene and ITS rRNA sequence, serve as good indicators of lineage separation (Erwin & Thacker, 2008; Osorio-Santos et al., 2014; Pietrasiak et al., 2019, 2021; Yarza et al., 2014; and many others). However, in isolation, we feel that these criteria should be used with more caution. When 16S rRNA gene similarity is below the microbiological thresholds for genus and species (94.5% and 98.7% respectively), this can serve as good evidence of lineage separation and justify description or recognition of separate taxa. However, these are arbitrary thresholds, and often taxa are encountered that straddle the threshold, as was the case shown in genera belonging to the Hapalosiphonaceae (Casamatta et al., 2020). This has also been observed with species, which often straddle the 16S rRNA gene threshold as well (Osorio-Santos et al., 2014). The ITS rRNA region thresholds seem to work well because there is often a marked discontinuity between intraspecific dissimilarity and interspecific dissimilarity (Osorio-Santos et al., 2014; Pietrasiak et al., 2019, 2021).

The use of ITS rRNA region data is especially problematic due to the presence of multiple ribosomal operons with ITS rRNA regions showing minor to major differences. When strains of cyanobacteria in the same genus are compared, if all operons are not obtained, then there is no way to know if differences between sequences are due to the presence of different species or the presence of multiple operons within the same species. This question arose in consideration of *Albertania latericola* and *Albertania egbensis*, both isolated from the same sample. Multiple operons have not yet been identified in any *Albertania* species, but it was considered a possibility in the case of these two species that lived in such close proximity. The deciding factor for the descriptions of both was the fact that the 16S rRNA gene similarity was 97.7%, well below the 98.7% threshold. So far, multiple operons with divergent ITS rRNA region sequences have 16S rRNA gene similarities that are almost identical, so the ITS rRNA region data and 16S rRNA gene data both indicate two species of *Albertania* were indeed living at the same site. Our *Egbenema* species also had low 16S rRNA gene similarity with each other, but this is almost an exception rather than the rule (Table 1). Often 16S rRNA gene data are insufficient for recognizing species, and it is the ITS rRNA region data that show lineage separation (Baldarelli et al., 2022; Becerra-Absalón et al., 2020; Johansen et al., 2014; Jung et al., 2020, 2022).

Although we often rely on 16S rRNA gene similarity below 98.7% and ITS rRNA region dissimilarity above 7% as good indicators of lineage separation that can be used to justify recognizing different species, we do not advocate the use of these thresholds to conclude that strains above or below these two thresholds indicate same species, at least not in isolation of other character sets such as morphology and ecology. The polyphasic approach is strong only when all available data are considered and weighted heavily. Following an evolutionary species concept, all clonal populations could be considered separate species, but most researchers would conclude that not all evolutionary species are worth taxonomic recognition (Mishler & Theriot, 2000). The monophyletic species concept provides some pragmatic criteria for recognizing species (Johansen & Casamatta, 2005). When all character sets (morphology, ecology, 16S RNA gene similarity and phylogeny, ITS rRNA region dissimilarity and phylogeny, and ITS rRNA region secondary structure analysis) do not provide evidence of lineage separation, then one might be dealing with the same species. When data are incomplete, then we can assume that taxa could be the same, if differences are not evident. With additional evidence, we may find they are different, but in the meantime, we can report that they are the same. This becomes a critical approach in metagenomics, where data are always incomplete. With smaller segments of the 16S rRNA gene, we can hypothesize species are present, but we should be aware that species or genera recognized in this way are likely inaccurately assigned. Metagenomic or ecogenomic approaches will continue to be problematic in comparison to the polyphasic approach.

The Nigerian cyanobacterial flora

With the small amount of phycological research, both floristic and molecular, that has been done in Nigeria, there is much work to be done, and we anticipate that many more species and genera will be described. From our unpublished work on the project that produced this manuscript, we have isolated new species of *Nodosilinea*, *Tildeniella*, *Oculatella*, and *Arthronema* that will be described in subsequent papers. These genera are all recently established genera with few species, but many "dark taxa" belong to them in NCBI GenBank. The Nigerian soils and subaerial habitats are rich in new species, particularly in the Synechococcales sensu Komárek et al. (2014). We only isolated strains from eight soil/subaerial samples in a geographically limited area and from sites with heavy anthropogenic impact. Much more sampling, isolation, and sequencing is required and will likely yield very high novel and

previously unknown diversity in this region of the world. Nigeria presents enormous opportunities for discovery, and collaborations with Nigerian researchers and cyanobacterial taxonomists in other parts of the world would undoubtedly be very fruitful. The same can probably be said for much of the African continent.

AUTHOR CONTRIBUTIONS

Mildred U. Akagha: Conceptualization (equal); investigation (lead); project administration (supporting); writing – original draft (lead). **Nicole Pietrasiak:** Investigation (supporting); resources (supporting); writing – review and editing (supporting). **David F. Bustos:** Investigation (supporting). **Alžběta Vonadášková:** Investigation (supporting); resources (supporting). **Sandra C. Lamb:** Investigation (supporting). **Jeffrey R Johansen:** Conceptualization (equal); formal analysis (lead); investigation (supporting); project administration (lead); resources (equal); supervision (lead); writing – review and editing (lead).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Uncollapsed Bayesian Inference phylogeny based on 427 partial sequences of the 16S rRNA gene. Nodal support of posterior probabilities and bootstrap values from maximum likelihood analysis.

Table S1. Sample alignment of the ITS rRNA region in genera covered in this study. Helices are coded in blue highlight with basal clamps in green highlight, tRNA genes are highlighted in red. D2, D3, and D4 regions are highlighted in yellow, and the Box-A region is in gray. Only seven strains can form a V2 helix, and these are not marked in the table.

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