Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean)

Maria Cellamare, Charlotte Duval, Yannick Drelin, Chakib Djediat, Nour Touibi, Hélène Agogué, Christophe Leboulanger, Magali Ader, Cécile Bernard

▶ **To cite this version:**

Maria Cellamare, Charlotte Duval, Yannick Drelin, Chakib Djediat, Nour Touibi, et al.. Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean). FEMS Microbiology Ecology, 2018, 94 (8), pp.fiy108. 10.1093/femsec/fiy108 . hal-01903434v2

HAL Id: hal-01903434
https://hal.science/hal-01903434v2

Submitted on 25 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean)

Cellamare M.[1,2], Duval C.[1], Drelin Y.[1], Djediat C.[1,3], Touibi N.[1,3], Agogué H.[4], Leboulanger C.[5], Ader M.[6], Bernard C.[1*]

[1] UMR7245 MCAM MNHN-CNRS, Muséum National d'Histoire Naturelle, CP 39, 12 rue Buffon, F-75231 Paris Cedex 05, France.

[2] Phyto-Quality, 15 rue Pétrarque, 75116 Paris, France.

[3] Electron Microscopy Platform, Muséum National d'Histoire Naturelle, CP 39, 12 rue Buffon, F-75231 Paris Cedex 05, France.

[4] UMR 7266 LIENSs CNRS-Univ. La Rochelle, F-17000 La Rochelle, France.

[5] UMR 9190 MARBEC IRD-CNRS- Univ. Montpellier-IFREMER, Avenue Jean Monnet, F-34200 Sète, France.

[6] Institut de Physique du Globe de Paris – Sorbonne Paris Cité, Univ. Paris Diderot, UMR 7154 CNRS, F-75005 Paris, France.

* Corresponding author. Tel.: +33 (0)140 793 183; Fax: +33 (0)140 793 594. E-mail address: cbernard@mnhn.fr (C. Bernard).

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

ABSTRACT

The saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean) is dominated by the bloom-forming cyanobacterium *Arthrospira*. However, the rest of the phototrophic community remains underexplored because of their minute dimension or lower biomass. To characterize the phototrophic microorganisms living in this ecosystem considered as a modern analog of Precambrian environments, several strains were isolated from the water column and stromatolites, and analyzed using the polyphasic approach. Based on morphological, ultrastructural and molecular (16S rRNA gene, 18S rRNA gene, 16S-23S ITS region, *cpc*BA-IGS locus) methods, seven filamentous cyanobacteria and the prasinophyte *Picocystis salinarum* were identified. Two new genera and four new cyanobacteria species belonging to the orders Oscillatoriales (*Desertifilum dzianense* sp. nov.) and Synechococcales (*Sodalinema komarekii* gen. nov., sp. nov., *Sodaleptolyngbya stromatolitii* gen. nov., sp. nov. and *Haloleptolyngbya elongata* sp. nov.) were described. This approach also allowed to identify *Arthrospira fusiformis* with exclusively straight trichomes instead of the spirally coiled form commonly observed in the genus. This study evidenced the importance of using the polyphasic approach to solve the complex taxonomy of cyanobacteria and to study algal assemblages from unexplored ecosystems.

Keywords: cyanobacteria, microalgae, crater-lake, saline-alkaline lake, polyphasic approach, *Arthrospira*

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

INTRODUCTION

Soda lakes are highly alkaline environments (pH typically between 9 and 12) characterized by large amounts of sodium carbonate (or complexes of this salt) formed by evaporative concentration (Jones and Grant 1999; Schagerl and Renaut 2016). Many soda lakes also contain high concentrations of sodium chloride and other dissolved salts, making them saline- or hypersaline systems (Grant 2006). The resulting high alkalinity and salinity make these lakes ones of the most extreme aquatic environments on Earth (Schagerl and Renaut 2016), where only alkaliphilic, halo- and thermotolerant organisms can thrive (Krienitz and Schagerl 2016). In saline-alkaline lakes from tropical and subtropical areas, the alkaliphilic filamentous cyanobacterium *Arthrospira* is frequently the dominant taxon and the responsible of permanent or seasonal blooms (Ballot *et al.* 2005; Sili, Torzillo and Vonshak 2012). However, the rest of the phototrophic microorganisms are often neglected because of their minute dimension or lower biomass. In consequence, their diversity remains unexplored and many taxa are still unknown. One of the main reasons to explain the low cyanobacterial and microalgal diversity usually reported from these systems relies on the use of traditional methods such as light–microscopy, that can underestimates true species number (Schagerl *et al.* 2015).

In the last decades, the polyphasic approach that combines phenotypic, molecular, ultrastructural, physiological and ecological characteristics has been commonly applied to unravel the complex taxonomy of cyanobacteria (Nübel, Garcia-Pichel and Muyzer 2000; Abed, Garcia-Pichel and Hernández-Mariné 2002; Suda *et al.* 2002; Casamatta *et al.* 2005; Taton *et al.* 2006; Comte *et al.* 2007; Řeháková *et al.* 2007; Dadheech *et al.* 2014; Komárek *et al.* 2014; Komárek 2016) and microalgae (Luo *et al.* 2010; Krienitz *et al.* 2012). This approach allowed to enhance the diversity of microorganisms, mostly by revealing previously overlooked taxa (Abed *et al.* 2003). For example, the cyanobacterial and microalgal diversity from saline-alkaline lakes distributed in the East African Rift valley has been well characterized using this method (Ballot, Dadheech and Krienitz 2004; Dadheech *et al.* 2012b; Krienitz *et al.* 2012; Dadheech *et al.* 2013; Krienitz, Dadheech and Kotut 2013). The isolation and characterization of phototrophic microorganisms living in saline-alkaline lakes are of great importance to solve taxonomic questions and contribute to the knowledge about their local environment, geographical distribution, ecology, physiology and behavior (Taton *et al.* 2006; Andreote *et al.* 2014).

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Dziani Dzaha is a small saline-alkaline crater-lake located in Mayotte Island (Comoros Archipelago, Western Indian Ocean) and situated very close to the ocean (0.1 km). Because Dziani Dzaha means "lake of the volcano" in shimaore, we refer to the lake as Dziani Dzaha in order to avoid redundancy. The physical and chemical characteristics of this lake are significantly different from other tropical soda lakes, mainly by its relatively balanced Na+ / Cl- composition that suggests significant contribution of seawater inputs (Leboulanger *et al.* 2017). Besides its high salinity (>50 psu) and alkalinity (>10 g L^{-1} CO_3^{2-}), this lake also presents a number of analogies with Precambrian environments as the permanent anoxia in depth, the presence of numerous stromatolites and a trophic chain mostly reduced to prokaryotes (Cadeau 2017; Leboulanger *et al.* 2017). Similar to other saline-alkaline lakes, the water column is characterized by cyanobacteria blooms dominated by *Arthrospira*, but the bloom appears permanent in Dziani Dzaha (Leboulanger *et al.* 2017).

Despite the extremely high algal biomass production all year round in Dziani Dzaha, only one study to date has focused on the microbial compartment (Leboulanger *et al.* 2017). Using the polyphasic approach, we characterize several strains of phototrophic microorganisms (cyanobacteria and microalgae) isolated from the water column and stromatolites of this unique ecosystem. Two new genera and four new species of filamentous cyanobacteria are described. Besides, ecological considerations regarding the morphological plasticity of several strains identified are highlighted.

MATERIALS AND METHODS

Study site

Dziani Dzaha is a tropical crater lake located in the Petite Terre of Mayotte Island (Comoros archipelago, Western Indian Ocean) (12°46’15”S; 45°17’17”E) (Fig. 1). The lake surface is close to 25 ha., with 600 m diameter and an altitude close to the mean sea level. It is a shallow lake with a maximum depth of 4.5 to 5.5 m depending on the rainfall. A pit of 18 m depth is located in the eastern part of the lake. The watershed is reduced to the crater walls, with no river inflow. The lake is chemically stratified in the dry season (May to October), forming two layers in the water column: an oxic layer (0-2.5 m) and an anoxic layer (>2.5 m). Over its entire area and depth, the water has high salinity (>50 psu), high alkalinity (dissolved inorganic carbon concentration in the range160-220 mM), conductivity between 77.1 and 79.7 mS cm^{-1} and pH about 9 to 9.5. The lake is very turbid (Secchi transparency <0.15 m) and the chlorophyll *a* concentrations are extremely high (up to 875 µg L^{-1}). Both parameters define

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

the hypereutrophic nature of this system. Water temperature is around 29 °C below 1 m, whereas surface temperatures show daily fluctuations, reaching up to 35 °C. Numerous stromatolites patches are distributed all around the lake shore and occasional gases bubbling occur on the surface (Leboulanger *et al.* 2017). More details about the lake characteristics are given in Leboulanger *et al.* (2017).

Sampling

Samples were collected for strains isolation in October 2010 (end of the dry season) and April 2014 (end of the rainy season). Water column samples were collected at the surface, with a 20-µm-mesh size net and benthic samples were collected directly from stromatolite biofilms (Fig. 1f).

Cyanobacterial and microalgal isolation and culture conditions

Water and stromatolite samples were inoculated on solid medium (5 or 10 g L^{-1} of agar) with several culture mediums: Z8-salt or Z8-salt minus nitrogen (Z8X) (Rippka 1988) or 0.2 µm filtered Dziani Dzaha raw water. Isolations were carried out by repeated transfers of single cells or filaments on solid or liquid media (at least 3 times) under an inverted microscope (Nikon ECLIPSE TS100). Growing clones were later cultured in 25 cm^2 culture flasks (Nunc, Roskilde, Denmark) containing 10 ml of Z8X-salt medium. Cyanobacteria strains were maintained in the Paris Museum Collection (PMC) and microalgae strains in the Algothèque Laboratoire Cryptogamie Paris (ALCP) at 25°C, using daylight fluorescent tubes providing an irradiance level of 16 µmol photons cm^{-2} s^{-1}, with a photoperiod of 16L: 8D. Strains and cultures were monoclonal and non-axenic.

Morphological analyses

Morphological analyses of cyanobacteria and microalgae strains were carried out using a light microscope NIKON Eclipse Ni and photographed with a coupled NIKON DS-Fi2 camera. The parameters analyzed included cell width and length, cell and filament morphology and motility presence. Strains identification by the traditional method was carried out using updated taxonomic literature (Lewin *et al.* 2000; Komárek and Anagnostidis 2005; Komárek 2007; Dadheech *et al.* 2012a; Dadheech *et al.* 2012b; Dadheech *et al.* 2014; Komárek and Johansen 2015).

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Ultrastructural analyses

Cyanobacterial and microalgal strains were analyzed by Transmission Electron Microscopy (TEM) as described by Parveen *et al.* (2013), with few modifications. Cells or filaments harvested from a growing culture by centrifugation were fixed with a mix of 2% glutaraldehyde, 2% formaldehyde, 0.18 M sucrose, 0.1% picric acid in 0.1 M, pH 7.4 Sorensen phosphate buffer (SPB) for 1h. The specimens were then washed three times with SPB and post fixed with 1% osmium tetraoxide for 1 h. Subsequently, they were washed with distilled water before being dehydrated in a graded ethanol series (30%, 50%, 70%, 90% and 100% ethanol), with agitation and centrifugation. The samples were embedded in EPON Resin and sectioned at 0.5 µm using an ultra-microtome (Reichert-Jung Ultracut) with a diamond knife and transferred onto 150-mesh copper grids. The prepared sections were stained with 2% uranyl acetate/50% ethanol for 15 min and washed three times in 50% distilled water/50% ethanol and finally twice in distilled water. The copper grids were then dried and examined using a transmission electron microscope (TEM, Hitachi HT-7700, Japan). Images were taken using a digital camera (Hamamatsu, Japan).

Molecular and phylogenetic analyses

DNA extraction from cyanobacteria and microalgae strains was carried out with a QiaGen kit (Cat. N° 69506) following manufacturer's protocol. Mechanical lysis was carried out through an ultrasonic cell probe Vibra Sonic (Granuloshop) for 30 s at a range of 100 % to 32.5 W. The amplification of the 16S rRNA gene, 16S-23S internal transcribed spacer region (ITS) and *cpc*BA-IGS locus was done with primers and PCR programs already described in Gugger and Hoffman (2004), Iteman *et al.* (2000) and Kumar *et al*. (2017), respectively.
Amplification of 18S rRNA gene was performed using the universal primer 515F (Caporaso *et al.* 2011) and the eukaryotic primer 951R (TTG-GYR-AAT-GCT-TTC-GC). All PCR reactions were carried out using the thermocycler Techne Echne FTC-Plus / 02. The PCR products were then sequenced by GENOSCREEN (Lille, France).
Sequences were assembled and corrected using ClustalW in MEGA7 version software (Kumar, Stecher and Tamura 2016). For 16S rRNA gene, a partial sequence of minimum 1 325 base pairs (bp) from the complete gene sequence (1 550 bp) was obtained. For 16S-23S ITS, *cpc*BA-IGS locus and 18S rRNA gene, partial sequences were obtained with a minimum of 460, 520 and 390 bp, respectively.
Phylogenetic analyses were performed according to three methods: neighbor joining (NJ), maximum likelihood (ML) and maximum parsimony (MP), using the MEGA7 version

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

software with an equal to 1 000 iteration. For 16S rRNA gene phylogeny, an overall alignment (n = 114) was generated, including the new produced sequences and those available in GenBank belonging to Oscillatoriales, Synechococcales and Spirulinales. The selected sequences were all longer than 1000 bp. *Gloeobacter violaceus* PCC 7421 (order Chroococcales) was chosen as outgroup. A cut-off value of 95% of 16S rRNA gene sequence identity was used for genus definition (Komárek 2010). The 18S rRNA gene phylogeny included the five ALCP strains and 26 sequences of GenBank. *Euglena gracilis* ASW 08025 was chosen as outgroup.

For *Arthrospira*, the 16S-23S ITS and *cpc*BA-IGS phylogenies were performed with four PMC *Arthrospira* strains (PMC 737.11, 738.11, 851.14, 894.15) and representative *Arthrospira* taxa (n = 41 and 33, respectively) from Genbank and Dadheech *et al.* (2010). *Phormidium autumnale* UTCC 579 and *P.* cf. *terebriformis* AB2002/07 served as outgroups, respectively. For *Desertifilum*, the 16S-23S ITS analysis included one PMC strain (PMC 872.14), six sequences of related taxa from previous studies (Dadheech *et al.* 2014; Cai *et al.* 2017) and seven sequences belonging to other taxa from GenBank. The 16S-23S ITS analysis of *Haloleptolyngbya* included three PMC strains (PMC 892.15, 893.15, 895.15), one sequence from Dadheech *et al.* (2012b) and six sequences from other taxa available in GenBank. *Gloeobacter violaceus* PCC 7421 (order Chroococcales) served as outgroup for both *Desertifilum* and *Haloleptolyngbya* 16S-23S ITS analyses. The generated 16S–23S ITS sequences were used for secondary structures analysis. The conserved regions (D1–D1', Box-B, V2 and V3) were analyzed using the Mfold WebServer (Zuker 2003). Default settings were used, except for the structure draw mode where natural angle was selected. tRNA genes were found using tRNAscan-SE 2.0 (Lowe and Chan 2016). The nucleotide sequences reported in this study have been deposited in the National Center for Biotechnology Information (NCBI) database. GenBank accession numbers are listed in the Supplementary table.

RESULTS

A total of 55 strains of cyanobacteria (50) and microalgae (5) were isolated from the water column and stromatolites from Dziani Dzaha. Two new genera and four new species of cyanobacteria were described from these strains. Cyanobacteria strains belonged to the orders Oscillatoriales: *Arthrospira fusiformis* (8 strains) and *Desertifilum dzianense* sp. nov. (12 strains); Synechococcales: *Sodalinema komarekii* gen. nov., sp. nov. (19 strains), *Sodaleptolyngbya stromatolitii* gen. nov., sp. nov. (2 strains), *Haloleptolyngbya alcalis* (2

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

strains), *Haloleptolyngbya elongata* sp. nov. (5 strains) and Spirulinales: *Spirulina subsalsa* (2 strains). The five microalgae strains were identified as the prasinophyte *Picocystis salinarum*. Identification numbers and habitat of all the strains are indicated in Table 1.

Morphological, ultrastructural and molecular characterization

Arthrospira fusiformis (Voronichin) Komárek *et* Lund

Arthrospira fusiformis was found as floating filaments, in mats covering the lake surface or attached to some stromatolites distributed on the edge of the lake. The thallus was blue-green and diffluent. Trichomes were solitary, olive green, isopolar, cylindrical, variously lengthened (170-2 390 µm), always straight (Fig. 2a-c) or slightly wavy (Fig. 2 d-e). Coiled trichomes were never observed. Trichomes were often gradually attenuated at the ends (Fig. 2a), not constricted at the cross-walls. Cells were always shorter than wide, 9-12 µm wide, 3-7 µm long. Cell content was granular with abundant aerotopes (Fig. 2c and d), mainly concentrated near the cross-wall (Fig. 3a-c). Sheaths were generally absent, but they were observed in some trichomes (Fig. 2e). Motility (gliding) was always present. The apical cell was rounded (Fig. 3d), often with thickened outer cell wall (Fig. 2b and c) and sometimes calyptrate (Fig. 2a-c). Cross-walls were visible under light microscope (Fig. 2d). Multiplication occurred by fragmentation of the trichome into hormogonia (Fig. 2f). The thylakoids showed a radial arrangement in the longitudinal and cross sections of the trichomes (Fig. 3a-f). The cells contained several cyanophycin granules (Fig. 3a-f) and carboxysomes (Fig. 3a, b and e). The septa between cells was strongly and regularly undulated (Fig. 3b and c). The 16S rRNA gene sequence analysis showed that *Arthrospira* strains from Dziani Dzaha shared ≥ 98% sequence identity with other *Arthrospira* strains (Fig. 4). The ITS region of *Arthrospira* strains comprised both $tRNA^{Ile}$ and $tRNA^{Ala}$ genes (data not shown). The phylogenetic tree of the 16S–23S ITS sequences of *Arthrospira* strains produced two main clusters (Fig. 5). The first cluster was mainly composed of strains of African or Indian origin (27 strains), four strains from Dziani Dzaha, two strains from other countries (Spain, Peru) and one strain of doubtful origin. The second cluster contained nine strains from the American continent (USA, Mexico, Peru), one strain from Thailand and one strain of doubtful origin. The D1-D1' secondary structure of *Arthrospira* strains from Dziani Dzaha was identical in terms of nucleotide number (62 nt) and sequence to those of African (*A. fusiformis* AY575929, Kenya) and Asian (*A. indica* PD1998/pus, India) strains (Fig. 6), while considerably different from those of American origin (GenBank no. FJ001892 and AJ292337). The phylogenetic tree of the *cpc*BA-IGS locus showed a similar pattern to that of the ITS tree and produced three clusters:

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

one big cluster (I) mainly containing strains of African or Indian origin (23 strains), the Dziani Dzaha strains (4 strains) and one strain of doubtful origin (Thailand?), and two small clusters (II and III) mainly containing strains from Mexico (Supplementary figure 1).

Desertifilum dzianense M. Cellamare, C. Duval, N. Touibi, C. Djediat et C. Bernard sp. nov. Strains of the genus *Desertifilum* were isolated from the stromatolites distributed on the edge of the lake. The thallus was dark green or blackish green, forming mats or empty balls in culture conditions. Filaments were solitary or entangled, more or less straight or wavy (Fig. 7a and b). Trichomes were cylindrical, pale blue-green, not or slightly constricted at cross-wall (Fig. 7a and b, 8a), with a colourless, thin and firm sheath attached to the trichome (Fig. 7b, 8a-d). Motility was present (slow gliding and oscillation). Cells were mainly longer than wide (rarely isodiametric), 2.4–3.4 µm wide, 3.4-5.9 µm long. The cell content was homogeneous, with numerous small granules and without aerotopes (Fig. 8a and b). The apical cell was conical-rounded (Fig. 7a and b, 8b), slightly hooked or bent (Fig. 7c), sometimes with extrusions (Fig. 7d). Cells contained numerous cyanophycin granules (Fig. 8a) and carboxysomes (Fig. 8c), and the thylakoids were parietally arranged in the peripheral zone (Fig. 8a and c). Comparison of 16S rRNA gene sequences revealed that the *Desertifilum* strains from Dziani Dzaha shared 99% of sequence identity with *D. tharense* and *D. salkalinema*, and 98% with *D. fontinale* (Fig. 4). The ITS region of the strains from Dziani Dzaha (with both $tRNA^{Ile}$ and $tRNA^{Ala}$ genes, data not shown) showed 98% of sequence similarity with *D. tharense* and *D. salkalinema*, and 94% with *D. fontinale* (Supplementary figure 2). Substantial differences were observed between the ITS secondary structures (D1-D1', V2 and V3 regions) of *Desertifilum dzianense* and the three *Desertifilum* species mentioned above (Fig. 9). The number of nucleotides of the D1-D1' region of *D. dzianense* was identical to that from *D. fontinale* (nt=57) but differed from *D. tharense* and *D. salkalinema* (nt=56). In terms of nucleotide sequence, the D1-D1' region of *D. dzianense* differed from the three other *Desertifilum* species in the terminal loop, with *D. fontinale* in the basal and side loops, and with *D. salkalinema* in the basal loop. Significant differences between the V2 region of *D. dzianense* and the other *Desertifilum* species were observed in the different portions in terms of sequence and number of nucleotides. Comparing the V3 secondary structure of *D. dzianense* with the other *Desertifilum* species, differences in the nucleotide sequence were observed in the terminal portion. Differences in the number of nucleotides with *D. fontinale* were also observed in the terminal portion of this region.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Formal description of the species:

Desertifilum dzianense M. Cellamare, C. Duval, N. Touibi, C. Djediat et C. Bernard sp. nov.

Diagnosis: Thallus dark green or blackish green, forming mats. Filaments solitary or entangled, more or less straight or wavy. Trichomes cylindrical, pale blue-green, not or slightly constricted at cross-wall, with a thin sheath colourless, motility present. Cells mainly longer than wide (rarely isodiametric), 2.4–3.4 µm wide. Cell content homogeneous, plenty of small granules. Apical cell conical-rounded, slightly hooked or bent, sometimes with extrusions. Aerotopes absent.

Holotype: a cryopreserved and formaldehyde-fixed sample of the strain PMC 872.14 deposited at Paris Museum Collection (PMC), Paris, France.

Type strain: a living strain deposited at Paris Museum Collection (PMC) under the no. PMC 872.14, Genbank MF579897.

Type locality: stromatolites, saline-alkaline environment, crater-lake Dziani Dzaha, Mayotte.

Etymology: the specific epithet (*dzianense*) refers to the name of the lake where the strain was isolated (Dziani Dzaha).

Leptolyngbya-like filaments

Strains with thin filaments (≤3 µm wide) were divided into four morphotypes (I, II, III, IV) on the basis of cell dimension, morphology and presence of aerotopes (Table 3).

Morphotype I

Strains of the morphotype I were found as floating filaments, in mats covering the lake surface or attached to some stromatolites. The thallus was emerald green, forming thin mats. These strains were characterized by filaments solitary, nearly straight, flexuous (Fig. 10a and b) or forming tight circular bundles in culture conditions (Fig. 10c-f). Trichomes were cylindrical, blue-green or yellow-green, slightly constricted at the cross walls (Fig. 11a and b) and very slightly attenuated at the ends (Fig. 11c). The trichomes sometimes possessed a very fine, attached and diffluent sheath (Fig. 11c, d and e). Motility was always present, with gliding in straight trichomes and rotation in spirally coiled trichomes. Cells were longer than wide (up to 2 times), 2.4-3 µm wide, 3.2-4.9 µm long, with a refractive granule (potentially a polyphosphate granule) on either or both sides of the cross-walls (Fig. 10a, 11a-d), cyanophycin granules (Fig. 11e) and carboxysomes (Fig. 11f). The apical cell was rounded, hemispherical and often possessed a large polar granule (aerotope?) (Fig. 10b and 11c). The thylakoids were parietally and concentrically arranged, parallel to the cell wall (10 to 11

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

layers) (Fig. 11a, b and f). Comparison of 16S rRNA gene sequences revealed that the morphotype I strains formed a separate clade with *Geitlerinema* from black band disease (BBD) of corals (93% of similarity) (Fig. 4).

Formal description of the genus and species:

Sodalinema komarekii M. Cellamare, C. Duval, N. Touibi, C. Djediat et C. Bernard gen. nov., sp. nov.

Diagnosis: Thallus emerald green, diffluent, forming thin mats. Filaments solitary, nearly straight, flexuous, blue-green or yellow-green. Trichome cylindrical, slightly constricted at the cross walls, very slightly attenuated at the ends, sometimes with a very fine, attached and diffluent sheath. Cells longer than wide, 2.4-3 µm wide. Cell content with a large refractive granule on either or both sides of the cross-walls. Apical cell rounded, hemispherical, often with a large granule (aerotope?). Motility present.

Holotype: a cryopreserved and formaldehyde-fixed sample of the strain PMC 869.14 deposited at Paris Museum Collection (PMC), Paris, France.

Type strain: a living strain deposited at Paris Museum Collection (PMC) under the no. PMC 869.14, GenBank MG772676.

Type locality: water column and stromatolites, saline-alkaline environment, crater-lake Dziani Dzaha, Mayotte.

Etymology: *Sodalinema*= the name of the genus refers to the alkaline-saline habitat where this cyanobacterium was found (soda lake) and to the cyanobacterium morphology (nema= filament); *komarekii*= named in honour of Prof. RNDr. Jiří Komárek, a Czech phycologist from the Institute of Botany, Academy of Sciences of the Czech Republic.

Morphotype II

The strains corresponding to the morphotype II were isolated from the stromatolites. The thallus was bright-green, forming thin, flattened and expanded mats. Filaments were solitary or entangled, nearly straight or slightly flexuous (Fig. 12a and b). Trichomes were thin, cylindrical, pale blue-green, not attenuated at the ends and slightly constricted at cross-walls (Fig. 12a, b and c). The sheath covering the trichome was attached, colourless and diffluent (Fig. 12c, d and e). Motility was present (gliding). Cells were always longer than wide (up to 2.9 times) (Fig. 12c), 1.5-2 µm wide, 1.9-4.8 µm long. The apical cell was rounded, without calyptra or thickened outer cell wall, and often presented a refractive granule (Fig. 12b), corresponding to numerous aerotopes (Fig. 12d). Cells contained a few cyanophycin granules and the thylakoids were parietally arranged, parallel to the cell wall (5-6 layers) (Fig. 12e and

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

f). Comparison of 16S rRNA gene sequences revealed that the morphotype II strains formed a single clade (Fig. 4).

Formal description of the genus and species:

Sodaleptolyngbya stromatolitii M. Cellamare, C. Duval, N. Touibi, C. Djediat et C. Bernard gen. nov., sp. nov.

Diagnosis: Thallus bright-green, forming thin, flattened and expanded mats. Filaments solitary or entangled, nearly straight or slightly flexuous. Trichomes thin, cylindrical, pale blue-green, not attenuated at the ends, slightly constricted at cross-walls, sheaths thin, attached, diffluent, colourless. Cells longer than wide, 1.5-2 µm wide. Apical cell rounded, with a refractive granule (numerous aerotopes with TEM). Motility present.

Holotype: a cryopreserved and formaldehyde-fixed sample of the strain PMC 867.14 deposited at Paris Museum Collection (PMC), Paris, France.

Type strain: a living strain deposited at Paris Museum Collection (PMC) under the no. PMC 867.14, GenBank MF579918.

Type locality: stromatolites, saline-alkaline environment, crater-lake Dziani Dzaha, Mayotte.

Etymology: *Sodaleptolyngbya* = the name of the genus refers to the alkaline-saline habitat where this cyanobacterium was found (=soda lake) and to the morphological similarity to members of the genus *Leptolyngbya*; *stromatolitii* = the specific epithet refers to the sampling environment (=stromatolite).

Morphotype III

Haloleptolyngbya alcalis Dadheech, Mahmoud, Kotut et Krienitz

Strains corresponding to the morphotype III were isolated from the water column. The thallus was downy, soft, forming mats, bright blue-green. Filaments were solitary or entangled, flexuous (Fig. 13a and b), sometimes wavy. Trichomes were pale blue-green, cylindrical, very constricted at cross-walls (Fig. 13a, c and d), not attenuated towards ends, consisting of very few (2) to several cells. Trichomes possessed a firm sheath with a thin transparent zone (Fig. 13c and d). Motility was not observed. Cells were isodiametric, shorter or longer than wide, barrel-shaped, 1.4-1.9 µm wide, 1.2-2.7 long, well separated from one another by cross-walls (Fig. 13c and d). The apical cell was conical-rounded, without calyptra or thickened outer cell wall (Fig. 13e). Thylakoids were parietally and concentrically arranged, parallel to the cell wall (5 layers) (Fig. 13c-f). Comparison of 16S rRNA gene revealed that the morphotype III strains were closely related to *Haloleptolyngbya alcalis* (GenBank no. JN712770.1) with 99% sequence similarity (Fig. 4). The ITS region of the morphotype III (with both $tRNA^{Ile}$ and

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

tRNA^{Ala} genes, data not shown) showed 98% similarity with *H. alcalis* (GenBank no. JN712771) (Supplementary figure 3). The ITS secondary structures (D1-D1', Box-B and V3 regions) of the morphotype III were almost identical to those of *H. alcalis* (Fig. 14). Although the D1-D1' region of the morphotype III showed a few differences with *H. alcalis* in number (6 and 11 nt instead of 7 and 10 nt, respectively) and sequence of nucleotides in the two terminal loops, the rest of the structure was conserved (Fig. 14). The Box-B and V3 regions were identical to those of *H. alcalis* in terms of number and sequence of nucleotides (Fig. 14).

Morphotype IV

Strains corresponding to the morphotype IV were isolated from the water column. The thallus was bright-green, forming mats. Filaments were solitary or entangled, straight or slightly arcuated, flexuous, sometimes undulated (Fig. 15a and b). Trichomes were pale blue-green, thin, cylindrical, consisting of very few (2) to several cells, very constricted at cross-walls (Fig. 15c), not attenuated towards the ends (Fig. 15b). A firm sheath with a thin transparent zone covered the trichome (Fig. 15c-f). Cells were cylindrical, always longer than wide (up to 3 times) (Fig. 15c and e), 0.9-1.5 µm wide, 1.5-3.9 µm long. The apical cell was conical-rounded (Fig. 15e), without calyptra or thickened outer cell wall. The cell content possessed polyphosphate granules (Fig. 15c and e). Motility was not observed. Thylakoids were parietally and concentrically arranged, parallel to the cell wall (4 layers) (Fig. 15c-f). Comparison of 16S rRNA gene sequences indicated that the strains of the morphotype IV were closely related to the *Haloleptolyngbya alcalis* (GenBank no. JN712770.1) cluster, but in a separated lineage, and shared 96% sequence similarity with this species (Fig. 4). The ITS sequence analysis (with both tRNA^{Ile} and tRNA^{Ala} genes, data not shown) revealed that the morphotype IV only shared 85% similarity with *H. alcalis* (GenBank no. JN712771) (Supplementary figure 3). ITS secondary structures analysis showed that the D1-D1' region of the morphotype IV (61 nt) differed from *H. alcalis* in the nucleotide sequence in both the terminal region and the main stem (Fig. 14). The Box-B region also showed substantial differences in terms of sequence and number of nucleotides (35 nt) with *H. alcalis* (49 nt). Only the V3 region was identical for both taxa in terms of sequence and number of nucleotides (23 nt).

Formal description of the species:

Haloleptolyngbya elongata M. Cellamare, C. Duval, N. Touibi, C. Djediat et C. Bernard sp. nov.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Diagnosis: Thallus bright-green, forming mats. Filaments solitary or entangled, straight or slightly arcuated, flexuous, sometimes undulated. Trichomes pale blue-green, thin, very constricted at cross-walls, not attenuated towards the ends, with a firm sheath with a thin transparent zone. Cells cylindrical, always longer than wide (up to 3 times), 0.9-1.5 µm wide. Apical cell conical-rounded. Motility was not observed.
Holotype: a cryopreserved and formaldehyde-fixed sample of the strain PMC 893.15 deposited at Paris Museum Collection (PMC), Paris, France.
Type strain: a living strain deposited at Paris Museum Collection (PMC) under the no. PMC 893.15, GenBank MF579910.
Type locality: water column, saline-alkaline environment, crater-lake Dziani Dzaha, Mayotte.
Etymology: the specific epithet (*elongata*) refers to the dimension of the cells which are up to 3 times longer than wide.

Spirulina subsalsa Oersted ex Gomont

Strains of *Spirulina subsalsa* were isolated from the stromatolites. The thallus was bright green and formed thin and flattened mats. Filaments were always entangled (Fig. 16a) Trichomes were blue-green, 1.6-2.2 µm wide, 50-287 µm long, regularly densely screw-like coiled (Fig. 16 b), straight or variously curved. Trichomes were rapidly motile, with screw-like rotation and simultaneous gliding. Cell content was homogeneous and the apical cell was rounded. Sequence analyses of the 16S rRNA gene and 16S-23S ITS region (with only the $tRNA^{Ile}$ gene, data not shown) confirmed that the strains from Dziani Dzaha belong to *Spirulina subsalsa* (96% and 98% of similarity, respectively).

Picocystis salinarum Lewin

Strains of *Picocystis salinarum* were isolated from the water column. Cells were green, generally globular to ellipsoidal, some were trilobate, 2.5-4.5 µm diameter (Fig. 17a and b). TEM observations showed a single nucleus in the cell and a lateral, usually cup-shaped and bilobed chloroplast (Fig. 17c, e and f), without pyrenoid but containing several starch grains (Fig. 17c). When cells were trilobate, the plastid was extending into two of the lobes and the nucleus into the third (Fig. 17c). A single elongated mitochondrion was located in the central cytoplasm, closely associated with the concavity of the chloroplast (Fig. 17c). Reproduction occurred by autosporulation leading to two daughter cells (Fig. 17d and e). Comparison of 18S rRNA gene sequences revealed that the strains from Dziani Dzaha shared 99% sequence identity with *P. salinarum* (GenBank no. KF615769.1) (Fig. 18).

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

DISCUSSION

This is the first study that characterizes the cyanobacteria and microalgae from Dziani Dzaha from strains isolated from the water column and stromatolites. This crater-lake is characterized by high salinity and alkalinity (Leboulanger *et al.* 2017) as many soda lakes in Eastern Africa (Ballot *et al.* 2005; Krienitz *et al.* 2012). These extreme environmental conditions lead to high productivity but low diversity of phototrophic microorganisms (Vonshak and Tomaselli 2000; Melack 2009), mostly dominated by *Arthrospira* (Tomaselli 1997; Sili, Torzillo and Vonshak 2012; Schagerl *et al.* 2015). In some way, the results obtained here agree with these findings since *Arthrospira* was the dominant taxon in Dziani Dzaha (>97% of the total biomass in all sampling occasions; Leboulanger *et al.* 2017). However, using the polyphasic approach, several filamentous cyanobacteria belonging to the orders Oscillatoriales, Synechococcales and Spirulinales, and the prasinophyte *Picocystis salinarum* were also identified.

Arthrospira strains from Dziani Dzaha shared several morphological characteristics with planktic *Arthrospira* species, including presence of aerotopes, cell dimension (width and length), apical cell rounded with thickened outer cell wall, sometimes calyptrate, trichome gradually attenuated at the ends, not constricted at the evident cross-walls and motility by gliding (Komárek and Anagnostidis 2005), However, the strains from Dziani Dzaha always presented straight trichomes. To the best of our knowledge, this is the first time that *Arthrospira* trichomes with constant straight morphology are observed in natural populations, which are typically characterized by spirally coiled trichomes (Vonshak and Tomaselli 2000; Komárek and Anagnostidis 2005) and only become straight under laboratory conditions or intensive cultures (Hindák 1985; Jeeji Bai 1985; Tomaselli 1997; Li, Debella and Carmichael 2001; Wang and Zhao 2005; Mühling *et al.* 2006; Noor *et al.* 2008; Sili, Torzillo and Vonshak 2012). The straight morphology of the trichomes isolated from Dziani Dzaha was conserved also under culture conditions.

The degree of coiling of the trichome has been considered as one of the main criteria to differentiate the two planktic *Arthrospira* species, the loosely coiled trichomes correspond to *A. maxima* and the tightly coiled trichomes to *A. fusiformis* (Komárek and Lund 1990; Li, Debella and Carmichael 2001; Mussagy, Wilmotte and Cronberg 2006). However, the taxonomical separation of these two species based on this feature is questionable due to the high variability in the degree of coiling of the trichomes (from straight to helical and vice

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

versa) observed in laboratory or culture conditions (Hindák 1985; Jeeji Bai 1985; Dadheech *et al.* 2010; Sili, Torzillo and Vonshak 2012). The finding of the straight morphotype of *Arthrospira* in Dziani Dzaha confirms that the coiled trichome criterion is unreliable to identify the planktic *Arthrospira* species. Indeed, this feature rendered difficult the identification of the material from Dziani Dzaha when only using the light microscope, but TEM observations provided several evidences to relate the studied strains with a planktic *Arthrospira* (e.g. radial thylakoids arrangement, presence of numerous aerotopes), later confirmed by the molecular analyses (≥ 98% 16S rRNA gene sequence similarity with other *Arthrospira* strains).

Results based on 16S–23S ITS region and *cpc*BA-IGS locus showed that *Arthrospira* strains from Dziani Dzaha were phylogenetically related to strains originating from Africa and Asia, that according to Dadheech *et al.* (2010) correspond to *A. fusiformis*. This result was confirmed by the ITS secondary structure (D1-D1') analysis that showed that *Arthrospira* strains from Dziani Dzaha possessed the same number and sequence of nucleotides than the *Arthrospira* strains from African and Asian origin. This genotypic conservation suggests that *Arthrospira* strains from Dziani Dzaha, Africa and Asia belong to a unique taxonomic unit. The use of diacritical (and more stable) features in the morphological and ultrastructural analyses, and the investigation of other gene loci or complete genome phylogenies will be necessary to resolve the complex taxonomy of this genus.

Soda lakes are often characterized by hidden diversity dominated by cyanobacterial taxa that possess simple morphology and lack of conspicuous traits (Komárek and Anagnostidis 2005; Andreote *et al.* 2014). This makes their identification problematic and as a result many taxa are still unknown or loosely assigned. Using the polyphasic approach, new taxa of filamentous cyanobacteria were characterized from Dziani Dzaha. Within the Oscillatoriales, a new *Desertifilum* species was identified. Comparison of 16S rRNA gene sequences revealed that strains of this cyanobacterium shared high sequence similarity (≥98%) with *Desertifilum tharense*, *D. fontinale* and *D. salkalinema*, those are the only three species of the genus until now described (Dadheech *et al.* 2012a; Dadheech *et al.* 2014; Cai *et al.* 2017). Considerable differences in the ITS secondary structures (D1-D1', V2 and V3 regions) allowed to discriminate the *Desertifilum* strains from Dziani Dzaha from the three other *Desertifilum* species. In addition to the phylogenetic analyses, morphological observations showed that the filaments from Dziani Dzaha were narrower than in *D. fontinale*, whereas the difference from

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

D. tharense and *D. salkalinema* mainly concerns the absence of gas vacuoles and the apical cell shape, respectively. Additionally, the ecology of the four taxa is different. While *D. tharense* was isolated from desert crusts (Thar Desert, Rajasthan, India) (Dadheech *et al.* 2012a), *D. fontinale* from free floating mats in a warm spring (near Lake Bogoria, Kenya) (Dadheech *et al.* 2014) and *D. salkalinema* from an *Arthrospira* culture pool (Zhejiang Province, China) (Cai *et al.* 2017), the *Desertifilum* strains from Dziani Dzaha were isolated from stromatolites. This study also showed that *Desertifilum* can thrive in ecosystems with higher salinity and alkalinity than those where the other members of the genus were found (Dadheech *et al.* 2012a; Dadheech *et al.* 2014; Cai *et al.* 2017). The habitat specificity of the strains studied here together with morphological, ultrastructural and molecular differences with the three *Desertifilum* mentioned above (Table 2) justify its classification as a new taxon. Thus, *Desertifilum dzianense* sp. nov. is described here as a benthic filamentous cyanobacterium associated to stromatolites from tropical saline-alkaline lakes.

The order Synechococcales was the most diversified group from Dziani Dzaha isolated strains and included four *Leptolyngbya*-like cyanobacteria. The taxonomic identification of members of the genus *Leptolyngbya* is very problematic due to its simple morphology and minute dimension that limit the number and strength of discriminating features (Komárek 2007). In consequence, the genetic diversity exceeds the morphological diversity in this genus (Albertano and Kováčik 1994; Casamatta *et al.* 2005; Komárek and Anagnostidis 2005; Johansen *et al.* 2008; Andreote *et al.* 2014; Komárek and Johansen 2015). The genus *Leptolyngbya* is defined by strains with very thin and simple trichomes (0.5–3.5 µm wide), sheaths present or facultative, without gas vesicles and peripherally arranged thylakoids (Komárek and Anagnostidis 2005; Komárek and Johansen 2015). These few morphological features are not discriminative enough to ensure a proper identification and as a consequence misidentifications often occur. For this reason, the different *Leptolyngbya*-like strains characterized in this study were separated in four morphotypes (I-IV) based onto morphological and ultrastructural features (Table 3).

Two of these morphotypes (I and II) were classified into new genera/species since they had very distinct morphological and ultrastructural characteristics and they were placed in single clusters in the phylogenetic tree based on 16S rRNA gene sequence data (Fig. 4). These two morphotypes possessed several morphological, ultrastructural and behavioral characters common with the genus *Leptolyngbya* (Komárek and Anagnostidis 2005; Komárek and

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Johansen 2015). However, apical cells in the strains from Dziani Dzaha always presented polar aerotopes or prominent granules and cells were always distinctly longer than wide, which according to Komárek and Johansen (2015) likely correspond to other genera. 16S rRNA gene sequence data showed that the strains of the morphotype I were unrelated to the clusters containing *Leptolyngbya* and formed a separate clade with *Geitlerinema* from black band disease (BBD) (93% sequence similarity). Since the absence of sheath is defined as a diacritical character in *Geitlerinema* (Komárek and Anagnostidis 2005), the strains from Dziani Dzaha could not be classified into this genus as a sheath was observed in some filaments. In addition, these strains were located phylogenetically distant from clusters containing *Geitlerinema sensu stricto* (that include *G. splendidum*, *G. carotinosum* and G. *pseudacutissimum*). This result also suggests that the *Geitlerinema* from BBD (GenBank no. 78064498, DQ151461.1) probably belongs to a different genus. The presence of sheath and the parietal and concentrically arranged thylakoids confirmed the affiliation of the strains from Dziani Dzaha to the order Synechococcales, family Leptolyngbyaceae (Komárek *et al.* 2014). Since the studied strains of this cyanobacterium could not be assigned to any known taxon, the new genus *Sodalinema* with the type species *S. komarekii* was established based on the phylogenetic position (16S rRNA gene sequences) and substantial differences in the phenotypic features (morphology and ultrastructure) with other genera of Leptolyngbyaceae. Interestingly, straight and dense spirally coiled filaments were observed within the same strains of this cyanobacterium. Although the occurrence of both morphologies in one genotype is not frequent (Komárek com. pers.), this phenomenon was also observed in *Leptolyngbya borchgrevinkii* from Antarctic marine waters (Komárek 2007) and *Leptolyngbya tentaculiformis* from an Australian thermal spring (McGregor and Rasmussen 2008). The explanation for this morphological variability remains unknown.

The lineage of the morphotype II strains showed their distinctiveness from other cyanobacteria genera (boostrap value <50%). Although morphological and ultrastructural characteristics of the morphotype II correspond to the genus *Leptolyngbya*, the very distant position from *Leptolyngbya sensu stricto* clades (Fig. 4) and the presence of numerous aerotopes in the terminal cell suggest that it belongs to a new genus into the order Synechococcales. The strains from Dziani Dzaha also showed >6% 16S rRNA gene sequence divergence to its closest related taxa that also possess aerotopes in the terminal cell (*i.e. Limnothrix*). Based on these results, the morphotype II fulfill the two criteria required to characterize a new genus that include less than 95% genetic similarity combined with at least

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

one autapomorphic character (Komárek 2010). Thus, the strains of the morphotype II were classified into the new genus *Sodaleptolyngbya* with the type species *S. stromatolitii*, that was found only associated to the stromatolites.

Regarding the morphotype III, the results based on the morphological and ultrastructural features (cell dimension and shape, thylakoids arrangement) together with the gene sequence analyses (16S rRNA gene, 16S-23S ITS region and ITS secondary structures) demonstrated that it can be classified as *Haloleptolyngbya alcalis*. This finding confirmed the affinity of this species to the high salinity and alkalinity typically found in soda lakes (Dadheech *et al.* 2012b). The morphotype IV formed a closely (but separated) related lineage with *H. alcalis* cluster (Fig. 4). The cut-off value of 95% of 16S rRNA gene sequence identity suggested for genus definition (Komárek 2010) allowed to classify the morphotype IV into the genus *Haloleptolyngbya* (96% sequence similarity with *H. alcalis*). However, morphological and ultrastructural features indicated that this morphotype did not belong to the species *H. alcalis*. While the cells in *H. alcalis* are wider and barrel-shaped (Dadheech *et al.* 2012b, this study), the morphotype IV is characterized by cylindrical, longer and thinner cells. The 16S–23S ITS region and secondary structures analyses confirmed the distinction between both taxa. These results allowed to describe a new *Haloleptolyngbya* species, identified as *Haloleptolyngbya elongata* sp. nov., a planktic filamentous cyanobacterium from tropical saline-alkaline lakes. These findings highlighted the importance of using the polyphasic approach to solve the complex taxonomy of *Leptolyngbya*-like filaments and suggest the verification of the different *Leptolyngbya* strains that occur in different clades in the phylogenetic tree of 16S rRNA gene (Albertano and Kováčik 1994; Dadheech *et al.* 2012b).

Among the order Spirulinales, *Spirulina subsalsa* was identified in the strains isolated from Dziani Dzaha, whose environment corresponds well to the typical habitat of the species (marine biotopes, inland salty waters) (Komárek and Anagnostidis 2005). Although the phototrophic community was dominated by cyanobacteria, bi- and trilobate cells of the prasinophyte *Picocystis salinarum* were also identified. *Picocystis* was previously reported in ponds and lakes with high salinity in North America, Asia and Africa (Lewin *et al.* 2000; Hollibaugh *et al.* 2001; Fanjing *et al.* 2009; Krienitz *et al.* 2012; Krienitz and Schagerl 2016). According to Lewin *et al.* (2000), trilobate forms of *P. salinarum* occur in old, nutrient-depleted cultures. However, trilobate cells were found here in both culture and environmental conditions where no nutrient limitation occurred.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Based on the isolated strains, the phytoplankton diversity in Dziani Dzaha was similar to that found by several authors in other saline-alkaline lakes worldwide, mainly characterized by *Arthrospira fusiformis*, *Leptolyngbya*-like filaments, *Haloleptolyngbya alcalis* and *Picocystis salinarum* (Tomaselli 1997; Lewin *et al.* 2000; Dadheech *et al.* 2012b; Krienitz *et al.* 2012; Sili, Torzillo and Vonshak 2012; Dadheech *et al.* 2013; Andreote *et al.* 2014; Schagerl *et al.* 2015). Since microalgal and cyanobacteria studies on stromatolites are scarce (Stal 2000; Abed *et al.* 2003; Foster *et al.* 2009), many taxa still remain unknown. This was the case in this study, where apart from *Spirulina subsalsa*, which was already known to be associated to stromatolites (Abed *et al.* 2003), the other cyanobacteria isolated from this structures are reported here for the first time (*Arthrospira fusiformis*, *Desertifilum dzianense* sp. nov., *Sodalinema komarekii* gen. nov., sp. nov. and *Sodaleptolyngbya stromatolitii* gen. nov., sp. nov.). Stromatolites have a long fossil record and are considered among the oldest evidence of the first forms of life on earth (Knoll 1999). In modern environments, benthic cyanobacteria are thought to be responsible for the formation of these organosedimentary structures in terms of organic input and structural integrity (Abed *et al.* 2003), thus their polyphasic characterization from modern stromatolites in Dziani Dzaha could offer interesting clues about the primitive microbial assemblage on early life.

In this lake, only the top meter is photosynthetically active because of the high turbidity and low light penetration (Leboulanger *et al.* 2017). These conditions favor the dominance of cyanobacteria, that are the most low light-adapted group among photosynthetic organisms (Schwaderer *et al.* 2011). All the cyanobacteria taxa isolated from Dziani Dzaha possess straight or elongate morphology (= high surface:volume ratio) which allows to maximize the surface exposed to light (Naselli-Flores and Barone 2011) in this extremely turbid ecosystem. Straight filaments are also more susceptible to grazing than the coiled ones (Padisák, Soróczki-Pintér and Rezner 2003; Kaggwa *et al.* 2013) but the risk of being grazed in Dziani Dzaha seems to be low as no primary consumers as zooplankton, fishes or Lesser Flamingos have been identified so far (Leboulanger *et al.* 2017). The real factors leading to the morphological specificity of the strains characterized in this study should be explored in greater detail in the future. Furthermore, studies focusing on the environmental microbial diversity in relation with the geochemical processes will be helpful to understand the functioning of this lake.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

CONCLUSIONS

The polyphasic approach allowed to identify a higher number of taxa than those determined using only the morphological evaluation. The identification of new taxa in such extreme environment was possible because of the strains isolation effort. They could be further used as taxonomic references for metagenomic studies on samples from Dziani Dzaha and other soda lakes. The results obtained also revealed a number of open questions concerning the morphological variability of phototrophic microorganisms and the low pressure from predators under extreme environmental conditions.

ACKNOWLEDGEMENTS

The authors highly thanks Professor J. Komárek for the valuable comments for the description of the new cyanobacteria taxa. They also thanks P. Got, D. Jezequel, G. Sarazin, P. Cadeau and E. Fouilland for their support during prospecting campaigns on Dziani Dzaha, N. Villeriot for 18S rRNA gene phylogeny and R. Peiffer for preliminary MET studies. This work was supported by Agence Nationale de la Recherche (France), http://www.agence-nationale-recherche.fr (grant n° ANR-13-BS06-0001), and Total Corporate Foundation, project DZAHA (grant n° C001493). Thanks to the Deep Carbon Observatory community for informative discussions on the importance of characterizing the ecosystems related to volcanic CO_2 emissions. The authors also acknowledge the anonymous referees for providing valuable suggestions that significantly improved the quality of the manuscript.

AUTHOR CONTRIBUTIONS

CB, HA, MA, CL, CD and CB have worked on the design of the experiments. CD, YD, CD, NT and CB have performed the experiments. MC, CD, NT and CB have performed the data analysis. MC, CD and CB have written the paper. CB co-led the project with CL and MA.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

REFERENCES

Abed RMM, Garcia-Pichel F, Hernández-Mariné M. Polyphasic characterization of benthic, moderately halophilic, moderately thermophilic cyanobacteria with very thin trichomes and the proposal of *Halomicronema excentricum* gen. nov., sp. nov. *Arch Microbiol* 2002;**177**:361-70.

Abed RMM, Golubic S, Garcia-Pichel F *et al.* Characterization of microbialite-forming cyanobacteria in a tropical lagoon: Tikehau atoll, Tuamotu, French Polynesia. *J Phycol* 2003;**39**:862-73.

Albertano P, Kováčik L. Is the genus *Leptolyngbya* (Cyanophyte) a homogeneous taxon? *Algol Stud* 1994;**75**:37–51.

Andreote APD, Vaz MGMV, Genuário DB *et al.* Non-heterocytous cyanobacteria from Brazilian saline-alkaline lakes. *J Phycol* 2014;**50**:675-84.

Ballot A, Dadheech PK, Krienitz L. Phylogenetic relationship of *Arthrospira*, *Phormidium* and *Spirulina* strains from Kenyan and Indian waterbodies. *Algol Stud* 2004;**113**:37-56.

Ballot A, Krienitz L, Kotut K *et al.* Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful Algae* 2005;**4**:139-50.

Cadeau P. Cycles biogéochimiques du carbone, de l'azote et du soufre dans un analogue des océans précambriens : le lac Dziani (Mayotte) volume PhD Thesis University Sorbonne Paris Cité, 2017.

Cai F-F, Chen Y-X, Zhu M-L *et al. Desertifilum salkalinema* sp. nov. (Oscillatoriales, Cyanobacteria) from an alkaline pool in China. *Phytotaxa* 2017;**292**:262-70.

Caporaso JG, Lauber CL, Walters WA *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci USA* 2011;**108**:4516-22.

Casamatta DA, Johansen JR, Vis ML *et al.* Molecular and ultrastructural characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria). *J Phycol* 2005;**41**:421-38.

Comte K, Šabacká M, Carré-Mlouka A *et al.* Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. *FEMS Microbiol Ecol* 2007;**59**:366-76.

Dadheech P, Huda M, Kotut K *et al. Desertifilum fontinale* sp. nov. (Oscillatoriales, Cyanobacteria) from a warm spring in East Africa, based on conventional and molecular studies. *Fottea, Olomouc* 2014;**14**:129-40.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Dadheech PK, Abed RMM, Mahmoud H *et al.* Polyphasic characterization of cyanobacteria isolated from desert crusts, and the description of *Desertifilum tharense* gen. et sp. nov. (Oscillatoriales). *Phycologia* 2012a;**51**:260-70.

Dadheech PK, Ballot A, Casper P *et al.* Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S–23S ITS and phycocyanin operon sequences. *Phycologia* 2010;**49**:361-72.

Dadheech PK, Glöckner G, Casper P *et al.* Cyanobacterial diversity in the hot spring, pelagic and benthic habitats of a tropical soda lake. *FEMS Microbiol Ecol* 2013;**85**:389-401.

Dadheech PK, Mahmoud H, Kotut K *et al. Haloleptolyngbya alcalis* gen. et sp. nov., a new filamentous cyanobacterium from the soda lake Nakuru, Kenya. *Hydrobiologia* 2012b;**691**:269-83.

Fanjing K, Qinxian J, Jia E *et al.* Characterization of a eukaryotic picoplankton alga, strain DGN-Z1, isolated from a soda lake in Inner Mongolia, China. *Nat Resour Env Iss* 2009;**15**:185-9.

Foster JS, Green SJ, Ahrendt SR *et al.* Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. *ISME J* 2009:1-15.

Grant WD. Alkaline environments and biodiversity. In: Gerday C, Glansdorff N (eds.) *Extremophiles (Life under extreme environmental condition)*. Oxford, UK.: Unesco-EOLSS publishers, 2006.

Gugger MF, Hoffmann L. Polyphyly of true branching cyanobacteria (Stigonematales). *Int J Syst Evol Microbiol* 2004;**54**:349-57.

Hindák F. Morphology of trichomes in *Spirulina fusiformis* Voronichin from lake Bogoria, Kenya. *Algol Stud–Arch Hydrobiol* 1985;**71-38/39**:209-18.

Hollibaugh JT, Wong PS, Bano N *et al.* Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event. *Hydrobiologia* 2001;**466**:45-60.

Iteman I, Rippka R, Tandeau de Marsac N *et al.* Comparison of conserved structural and regulatory domains within divergent 16SrRNA–23S rRNA spacer sequences of cyanobacteria. *Microbiology* 2000;**146**:1275-86.

Jeeji Bai N. Competitive exclusion or morphological transformation? A case study with *Spirulina fusiformis*. *Algol Stud–Arch Hydrobiol* 1985;**71** 191-9.

Johansen JR, Olsen CR, Lowe RL *et al. Leptolyngbya* species from selected seep walls in the Great Smoky Mountains National Park. *Algol Stud* 2008;**126**:21-36.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Jones BE, Grant WD. *Microbial diversity and ecology of the Soda Lakes of East Africa*. Microbial Biosystems: New Frontiers: Proceedings of the 8th International Symposium on Microbial Ecology. Halifax, Canada: Atlantic Canada Society for Microbial Ecology, 1999.

Kaggwa MN, Burian A, Oduor SO *et al.* Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes. *MicrobiologyOpen* 2013;**2**:881-91.

Knoll AH. A new molecular window on early life. *Science* 1999;**285**:1025-6.

Komárek J. Phenotype diversity of the cyanobacterial genus *Leptolyngbya* in the maritime Antarctic. *Pol Polar Res* 2007;**28**:211–31.

Komárek J. Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). *Hydrobiologia* 2010;**639**:245-59.

Komárek J. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. *Eur J Phycol* 2016;**51**:346-53.

Komárek J, Anagnostidis K. *Cyanoprokaryota 2. Teil: Oscillatoriales. Süsswasserflora von Mitteleuropa 19/2*: Elsevier SpeKtrum Akademischer Verlag Edition. München: Elsevier, 2005.

Komárek J, Johansen JR. Filamentous cyanobacteria. In: Wehr JD, Sheath RG, Kociolek JP (eds.) *Freshwater algae of North America*. USA: Academic Press, 2015, 135-235.

Komárek J, Kaštovský J, Mareš J *et al.* Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* 2014;**86**:295-335.

Komárek J, Lund JWG. What is '*Spirulina platensis*' in fact? *Algol Stud* 1990;**58**:1-13.

Krienitz L, Bock C, Kotut K *et al. Picocystis salinarum* (Chlorophyta) in saline lakes and hot springs of East Africa. *Phycologia* 2012;**51**:22-32.

Krienitz L, Dadheech P, Kotut K. Mass developments of the cyanobacteria *Anabaenopsis* and *Cyanospira* (Nostocales) in the soda lakes of Kenya: ecological and systematic implications. *Hydrobiologia* 2013;**703**:79-93.

Krienitz L, Schagerl M. Tiny and Tough: Microphytes of East African Soda Lakes. In: Schagerl M (ed.) *Soda Lakes of East Africa*. Cham: Springer International Publishing, 2016, 149-77.

Kumar D, Dhar DW, Nehra P *et al.* Similarity analysis of *Spirulina*/*Arthrospira* strains on the basis of phycocyanin operon locus (cpcB-IGS-cpcA) and 16S rRNA gene sequences. *Indian Journal of Biotechnology* 2017;**16**:84-90.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 2016;**33**:1870-4.

Leboulanger C, Agogué H, Bernard C *et al.* Microbial diversity and cyanobacterial production in Dziani Dzaha crater lake, a unique tropical thalassohaline environment. *PLOS ONE* 2017;**12**:e0168879.

Lewin RA, Krienitz R, Goericke H *et al. Picocystis salinarum* gen. et sp. nov. (Chlorophyta) - a new picoplanktonic green alga. *Phycologia* 2000;**39**:560-5.

Li R, Debella HJ, Carmichael WW. Isolates identifiable as *Arthrospira maxima* and *Arthrospira fusiformis* (Oscillatoriales, Cyanobacteria) appear identical on the basis of a morphological study in culture and 16S rRNA gene sequences. *Phycologia* 2001;**40**:367-71.

Lowe TM, Chan PP. tRNAscan-SE On-line: Search and Contextual Analysis of Transfer RNA Genes. *Nucleic Acids Res* 2016;**44**:W54-7.

Luo W, Pröschold T, Bock C *et al.* Generic concept in *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). *Plant Biology* 2010;**12**:545-53.

McGregor GB, Rasmussen JP. Cyanobacterial composition of microbial mats from an Australian thermal spring: a polyphasic evaluation. *FEMS Microbiol Ecol* 2008;**63**:23-35.

Melack JM. Saline and soda lakes. In: Jørgensen SE (ed.) *Ecosystem Ecology*. Amsterdam, The Netherlands: Elsevier, 2009, 380-4.

Mühling M, Somerfield PJ, Harris N *et al.* Phenotypic analysis of *Arthrospira* (*Spirulina*) strains (cyanobacteria). *Phycologia* 2006;**45**:148-57.

Mussagy A, Wilmotte A, Cronberg G. The cyanophyte *Arthrospira fusiformis* from Mozambique, Africa: morphological and molecular characterization. *Algol Stud* 2006;**121**:59-73.

Naselli-Flores L, Barone R. Fight on plankton! Or, phytoplankton shape and size as adaptive tools to get ahead in the struggle for life. *Cryptogam, Algol* 2011;**32**:157-204.

Noor P, Akhtar N, Munshi JL *et al. Spirulina* culture in Bangladesh XII. Effects of different culture media, different culture vessels and different cultural conditions on coiled and straight filament characteristics of *Spirulina*. *Bangladesh J Sci Ind Res* 2008;**43**:369-76.

Nübel U, Garcia-Pichel F, Muyzer G. The halotolerance and phylogeny of cyanobacteria with tightly coiled trichomes (*Spirulina* Turpin) and the description of *Halospirulina tapeticola* gen. nov., sp. nov. *Int J Syst Evol Microbiol* 2000;**50**:1265-77.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Padisák J, Soróczki-Pintér É, Rezner Z. Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton – an experimental study. *Hydrobiologia* 2003;**500**:243-57.

Parveen B, Ravet V, Djediat C *et al.* Bacterial communities associated with *Microcystis* colonies differ from free-living communities living in the same ecosystem. *Environmental Microbiology Reports* 2013;**5**:716-24.

Řeháková K, Johansen JR, Casamatta DA *et al.* Morphological and molecular characterization of selected desert soil cyanobacteria: three species new to science including *Mojavia pulchra* gen. et sp. nov. *Phycologia* 2007;**46**:481-502.

Rippka R. Isolation and purification of cyanobacteria. *Methods Enzymol* 1988;**167**:3-27.

Schagerl M, Burian A, Gruber–Dorninger M *et al.* Algal communities of Kenyan soda lakes with a special focus on *Arthrospira fusiformis*. *Fottea, Olomouc* 2015;**15**:245-57.

Schagerl M, Renaut RW. Dipping into the Soda Lakes of East Africa. In: Schagerl M (ed.) *Soda Lakes of East Africa*. Cham: Springer International Publishing, 2016, 3-24.

Schwaderer A, Yoshiyama K, de Tezanos Pinto P *et al.* Eco-evolutionary differences in light utilization traits and distributions of freshwater phytoplankton. *Limnol Oceanogr* 2011;**56**:589-98.

Sili C, Torzillo G, Vonshak A. *Arthrospira* (*Spirulina*). In: Whitton AB (ed.) *Ecology of Cyanobacteria II: Their diversity in space and time*. Dordrecht: Springer Netherlands, 2012, 677-705.

Stal LJ. Cyanobacterial mats and stromatolites. In: Whitton BA, Potts M (eds.) *The ecology of cyanobacteria: Their diversity in space and time*. The Netherlands: Kluwer Academic Publisher, 2000, 61-120.

Suda S, Watanabe MM, Otsuka S *et al.* Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. *Int J Syst Evol Microbiol* 2002;**52**:1577-95.

Taton A, Grubisic S, Ertz D *et al.* Polyphasic study of Antarctic cyanobacterial strains. *J Phycol* 2006;**42**:1257-70.

Tomaselli L. Morphology, ultrastructure and taxonomy. In: Vonshak A (ed.) *Spirulina platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology*. London, UK: Taylor and Francis, 1997, 1-15.

Vonshak A, Tomaselli L. *Arthrospira* (*Spirulina*): Systematics and ecophysiology. In: Whitton BA, Potts M (eds.) *The ecology of cyanobacteria*. Netherlands: Kluwer Academic Publishers, 2000, 505-19.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Wang ZP, Zhao Y. Morphological reversion of *Spirulina* (*Arthrospira*) *platensis* (Cyanophyta): from linear to helical. *J Phycol* 2005;**41**:622-8.

Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 2003;**31**:3406-15.

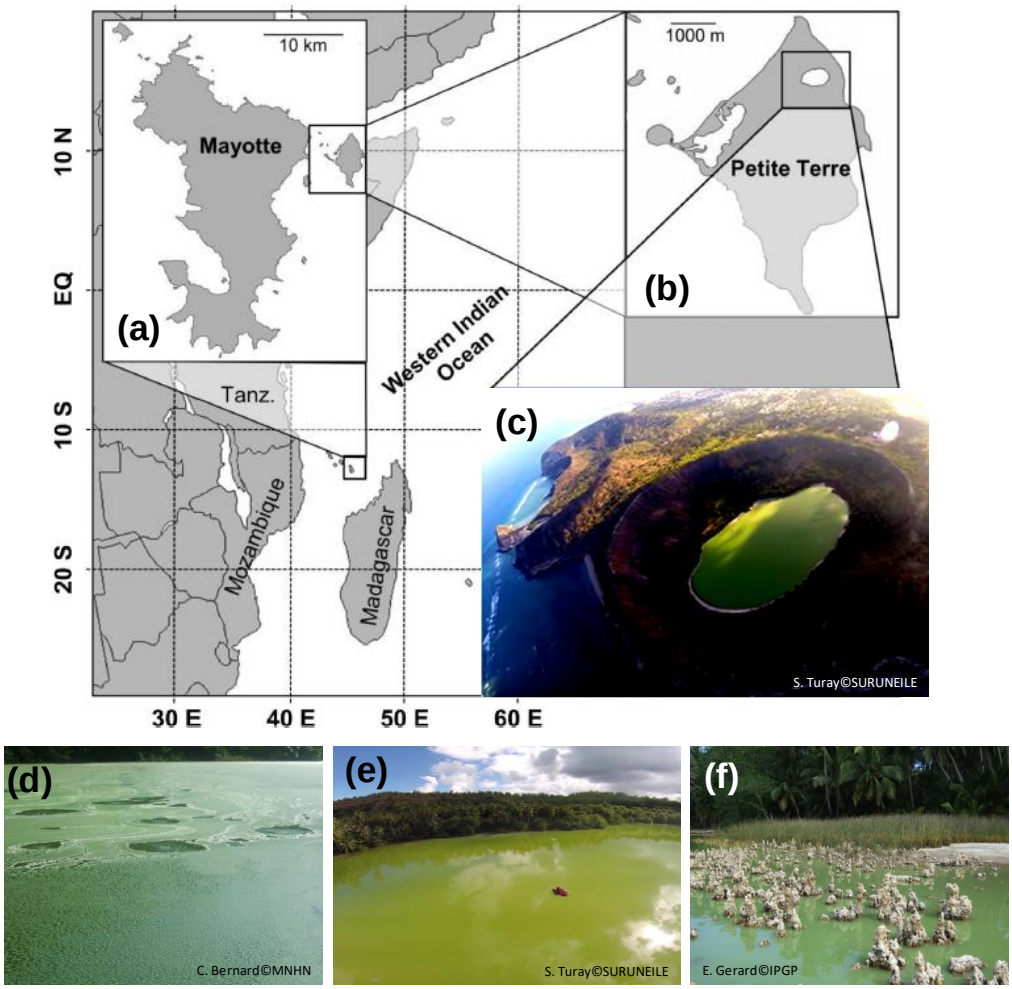

Figure 1. Location of Dziani Dzaha (Mayotte Island), situated in the northern Mozambique Channel in the Indian Ocean (a). Mayotte consists in a main island, Grande-Terre (a) and a smaller island, Petite-Terre (a, b). Dziani Dzaha is a round crater-lake in the Petite-Terre island (b, c) with two different biotopes: water column (d, e) and stromatolites (f).

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

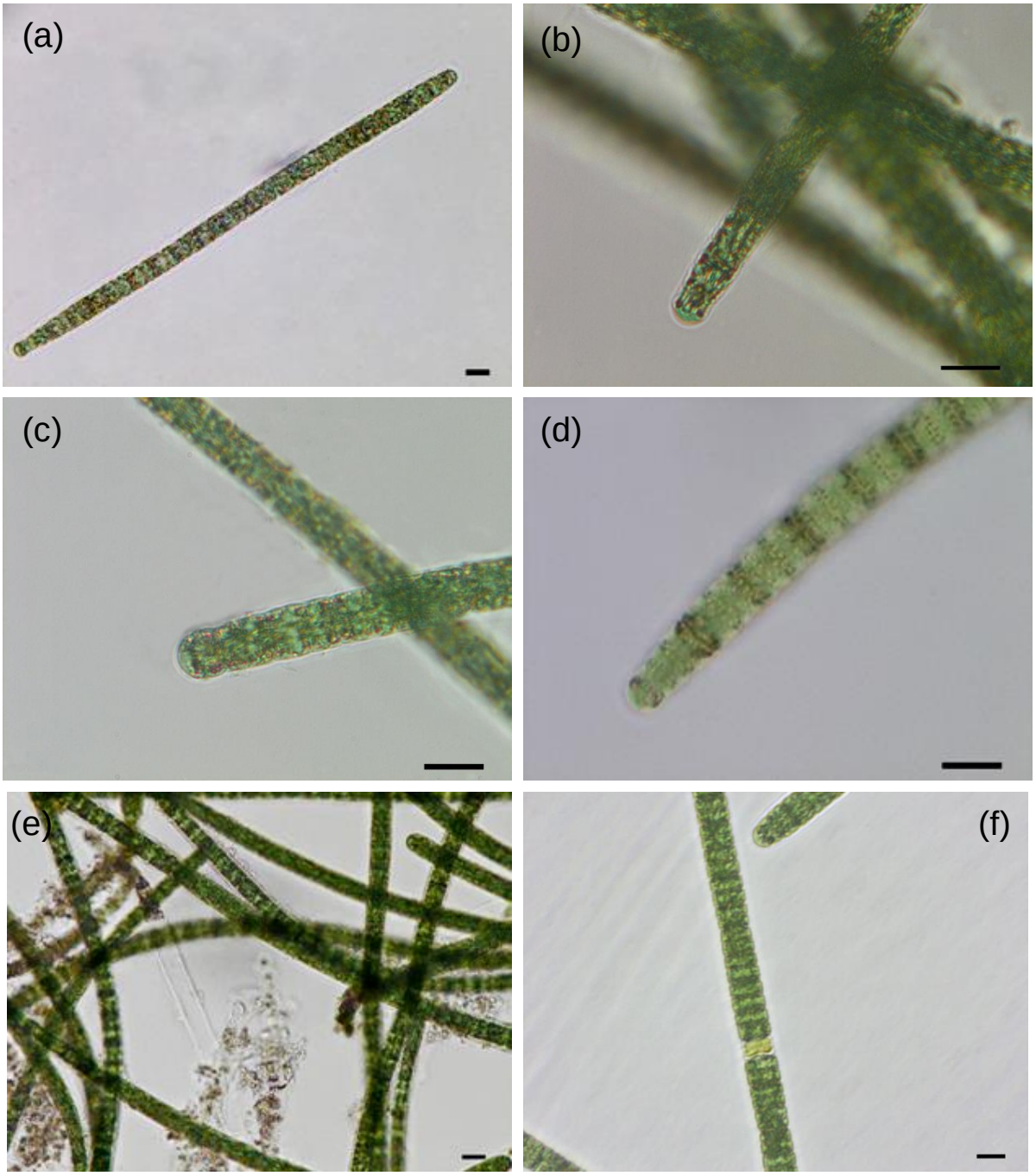

Figure 2. Light microscopy micrographs of *Arthrospira fusiformis* strains (PMC 851.14) from Dziani Dzaha. a: general view of a filament showing a gradual attenuation towards the ends; b-c: apical cell rounded with thickened outer cell wall and calyptrate; d: filament showing a very visible cross-wall; e: filament with sheath; f: filament with hormogonium. Scale bars: 10 µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

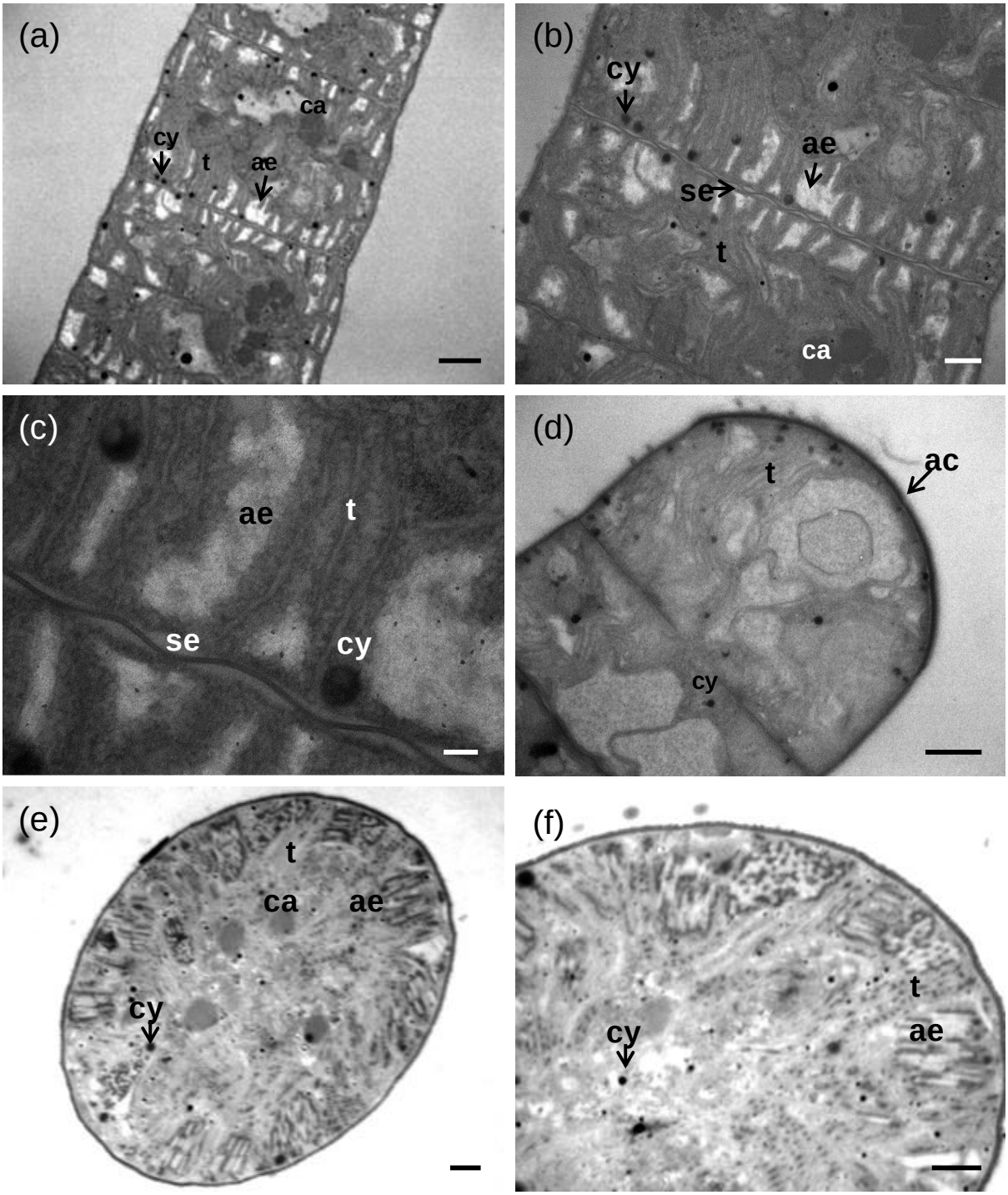

Figure 3. TEM micrographs of *Arthrospira fusiformis* strains (PMC 737.11, 851.14) from Dziani Dzaha. Longitudinal (a-d) and cross sections (e-f) of the trichome showing the ultrastructural details. Abbreviations: ac: apical cell, ae: aerotopes, ca: carboxysome, cy: cyanophycin granules, se: septum, t: thylakoids. Scale bars: c = 100 nm; b, e, f = 500 nm; a, d = 1µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

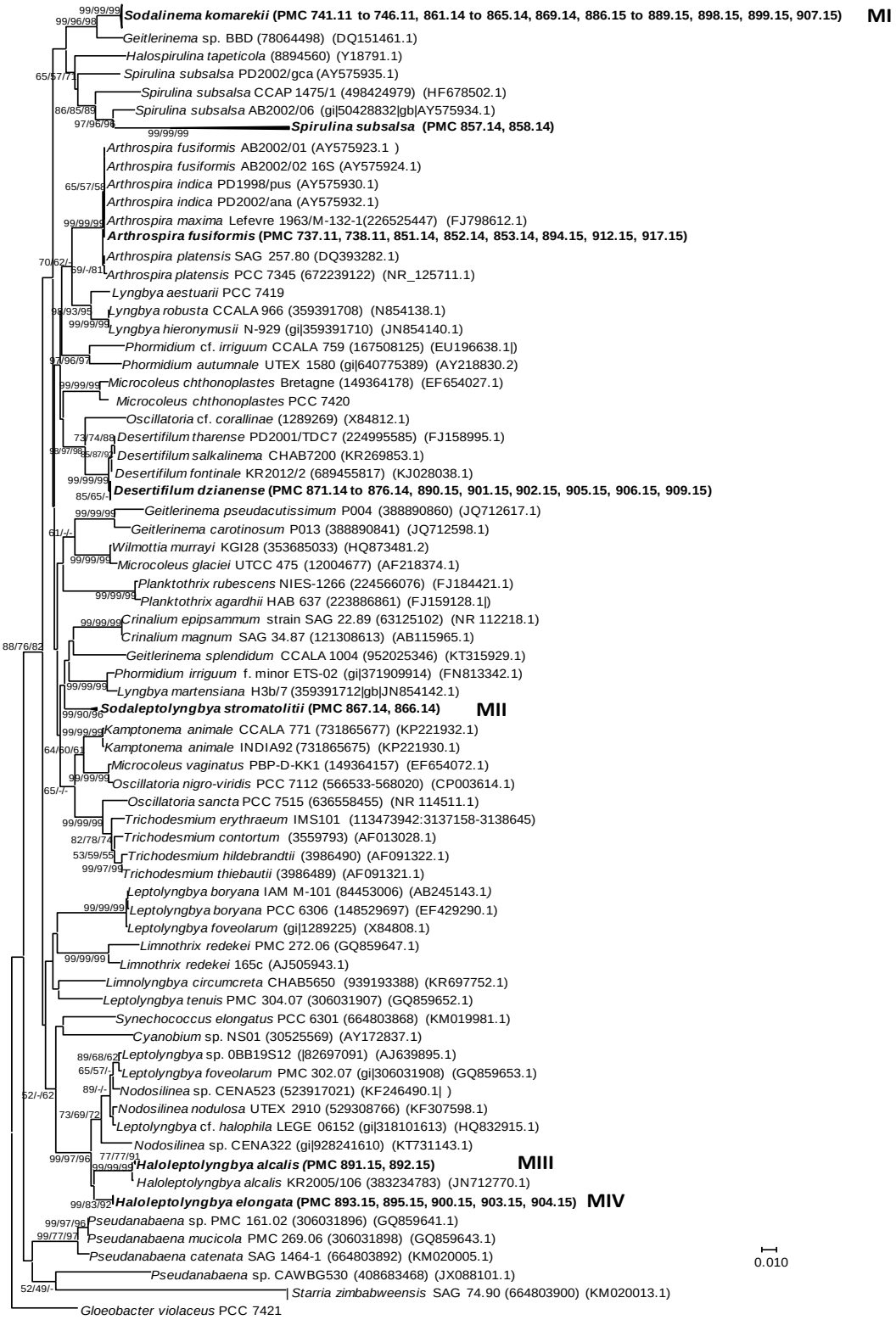

Figure 4. Consensus phylogenetic tree based on 16S rRNA gene sequences of representative cyanobacteria strains belonging to the orders Oscillatoriales, Spirulinales and Synechococcales, Dziani Dzaha strains (in bold) and one outgroup (*Gloeobacter violaceus*). Numbers above branches indicate bootstrap support (>50 %) from 1000 replicates. Bootstrap values are given in the following order: neighbor joining/maximum likelihood/maximum parsimony. M: morphotype.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

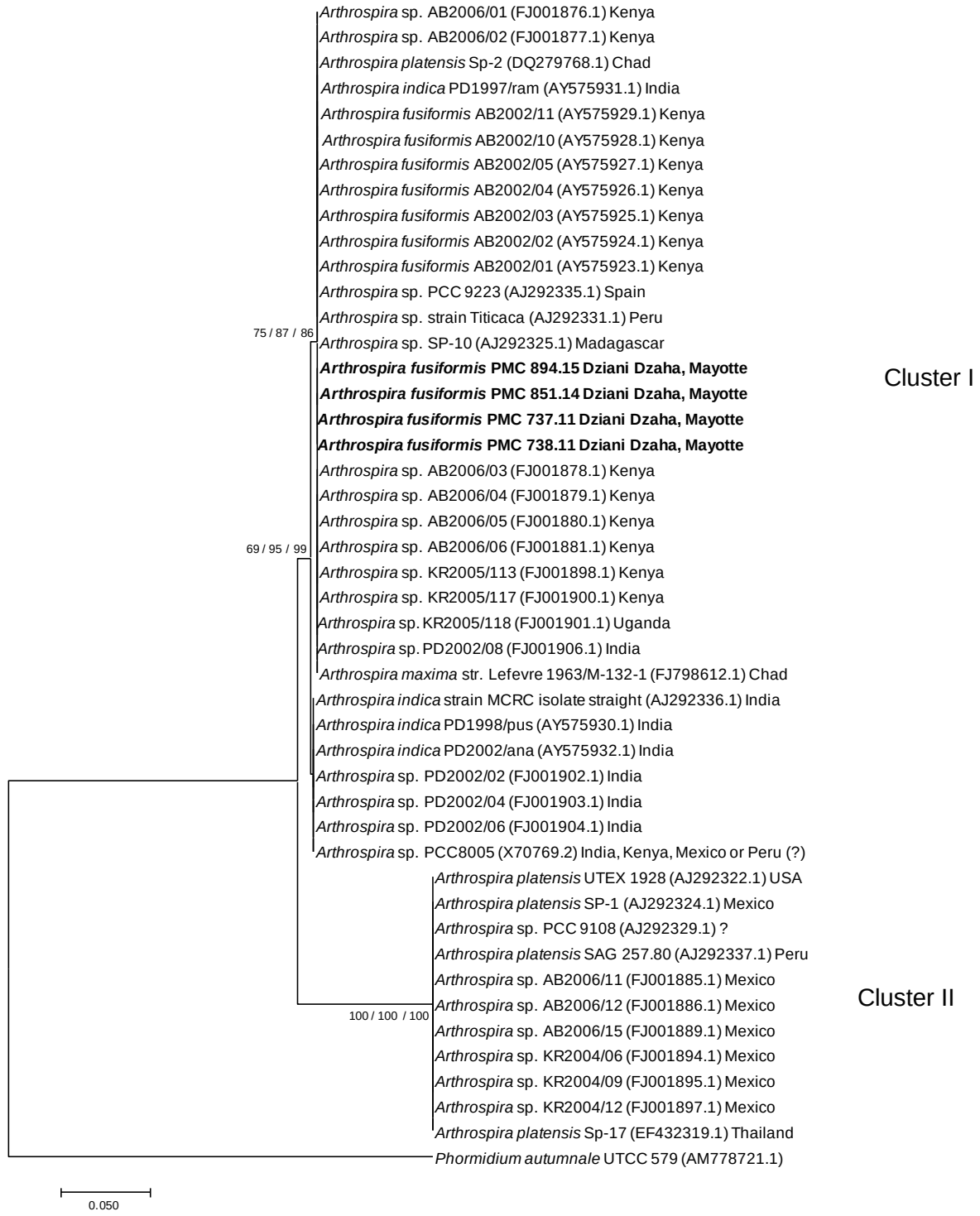

Figure 5. Consensus phylogenetic tree based on 16S–23S ITS sequences of *Arthrospira* strains. *Arthrospira* strains from Lake Dziani are shown in bold. The other strains used are from Dadheech *et al.* (2010) and GenBank. Bootstrap values are given in the following order: neighbor-joining/maximum likelihood/maximum parsimony. Only support values > 50% are shown.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

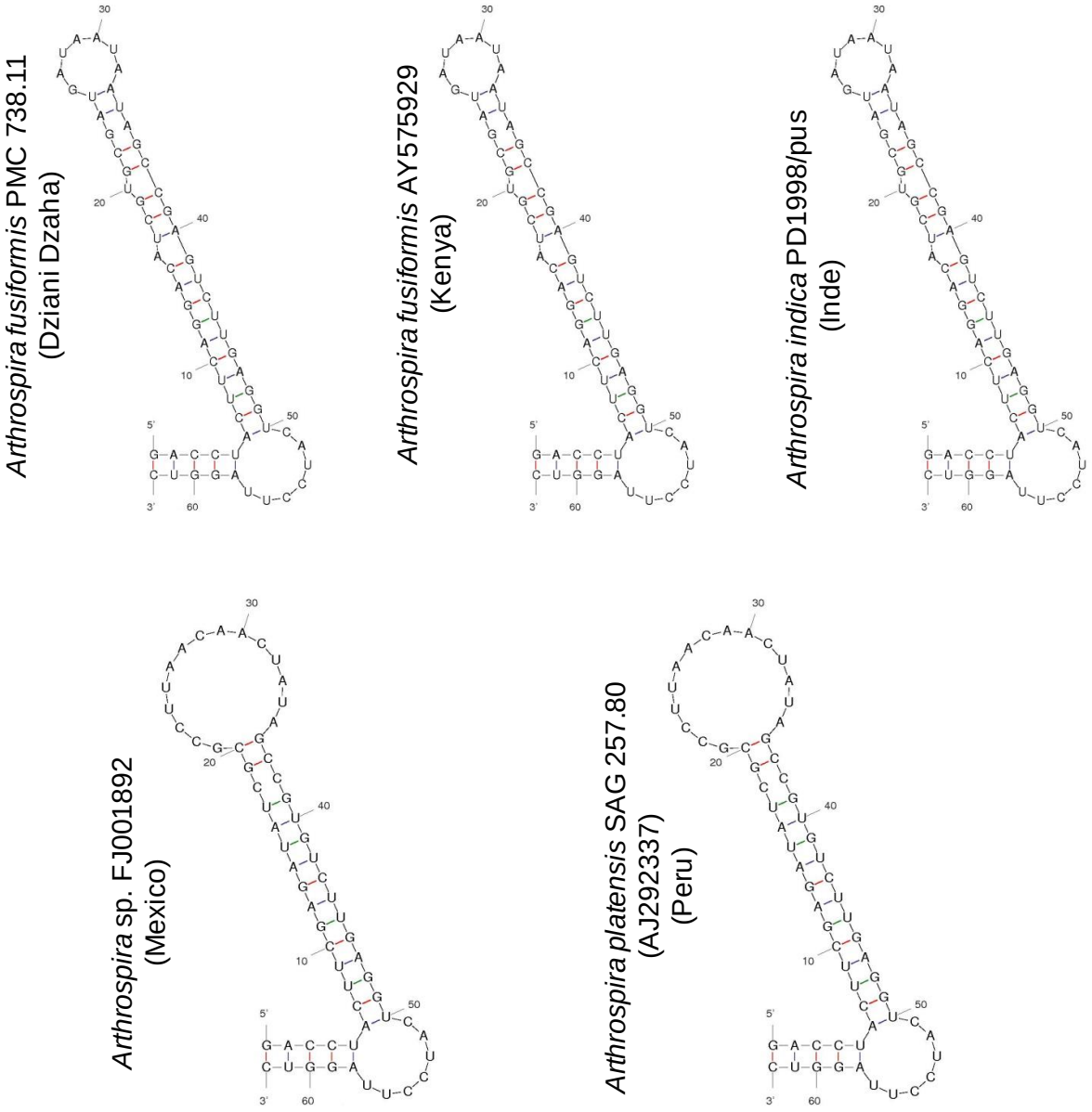

Figure 6. 16S-23S ITS secondary structure (D1-D1') of *Arthrospira* strains from Dziani Dzaha, Africa, Asia and America.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

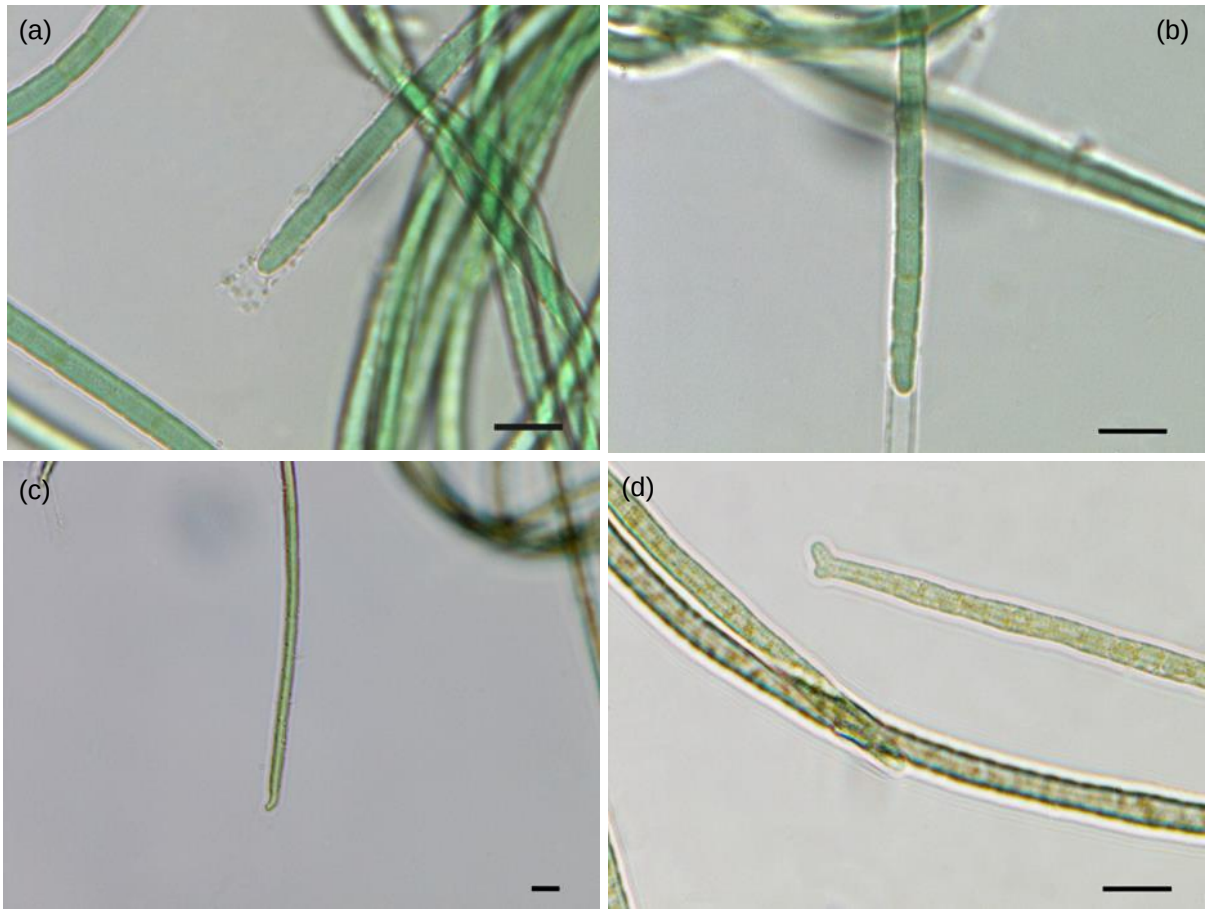

Figure 7. Light microscopy micrographs of *Desertifilum dzianense* strains (PMC 872.14) from Dziani Dzaha. a: image showing the entangled filaments, apical cell rounded and tapering end of a filament; b: a filament with sheath; c: filament with end cell bent; d: filament with extrusion. Scale bars: 10 µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

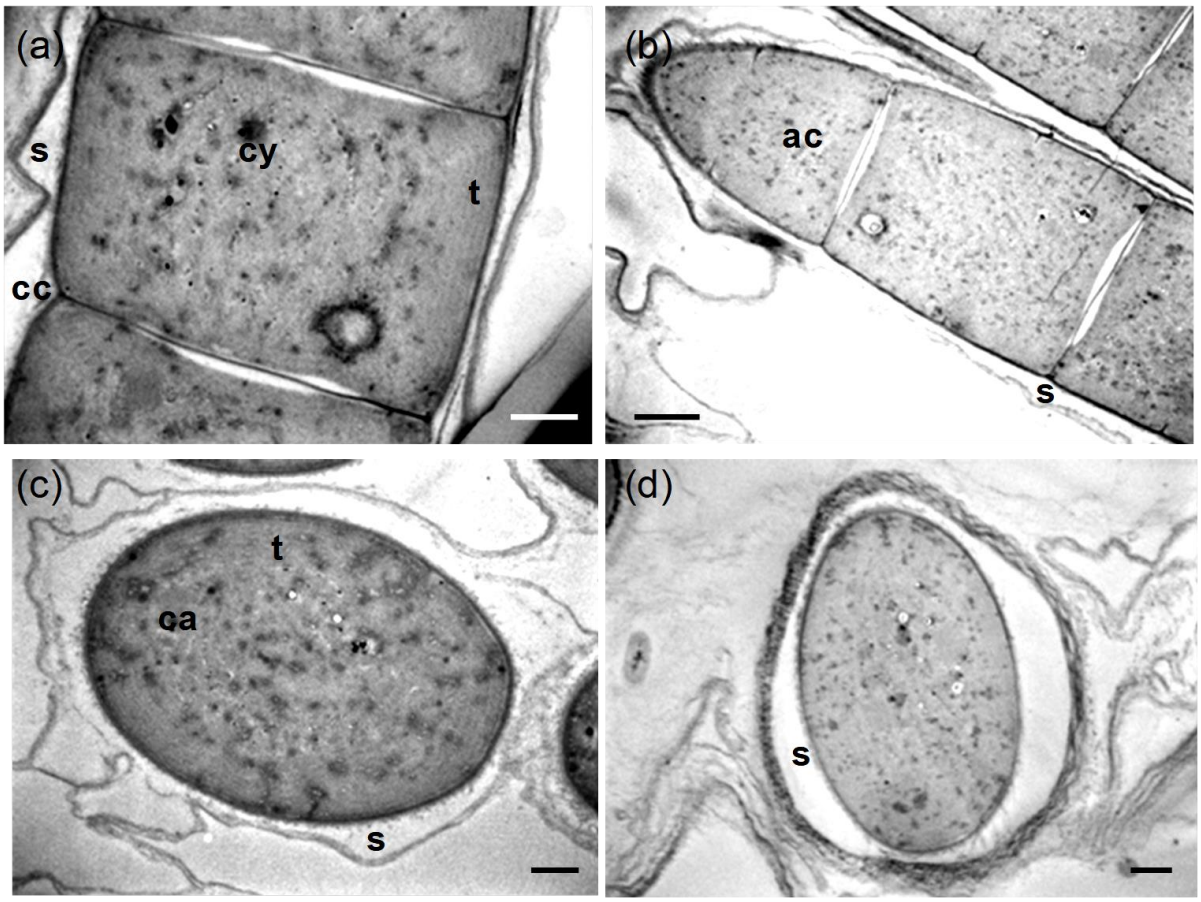

Figure 8. TEM micrographs of *Desertifilum dzianense* strains (PMC 872.14) from Dziani Dzaha. Longitudinal (a-b) and cross sections (d-e) of the trichome showing the ultrastructural details. Abbreviations: ac: apical cell, ca: carboxysome, cc: cross-wall constriction, cy: cyanophycin granules, s: sheath, t: thylakoids. Scale bars: a, c, d = 500 nm; b = 1µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

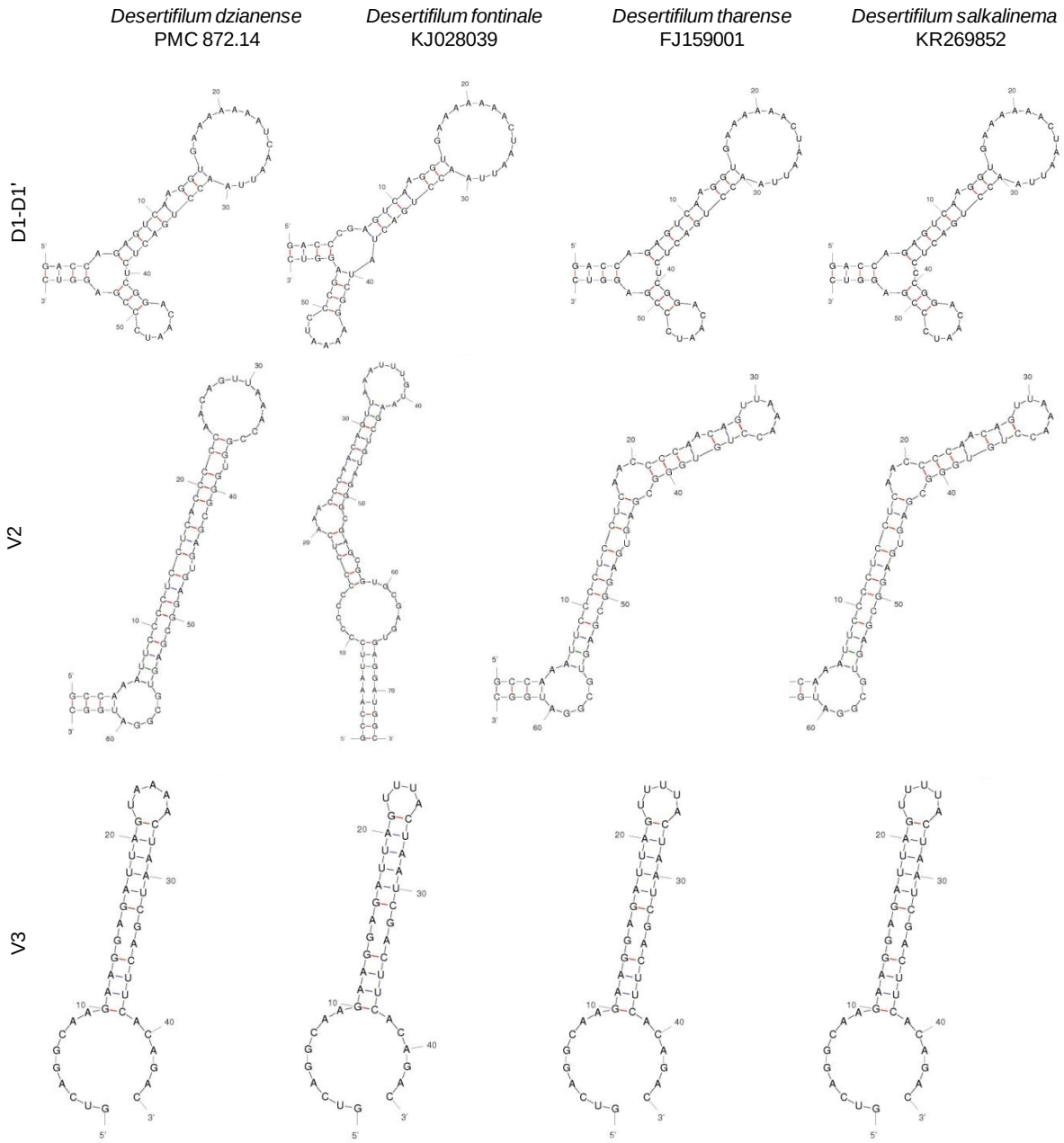

Figure 9. 16S-23S ITS secondary structures (D1-D1', V2 and V3) of *Desertifilum dzianense* from Dziani Dzaha and other *Desertifilum* species (Dadheech *et al.* 2012a; Dadheech *et al.* 2014; Cai *et al.* 2017).

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

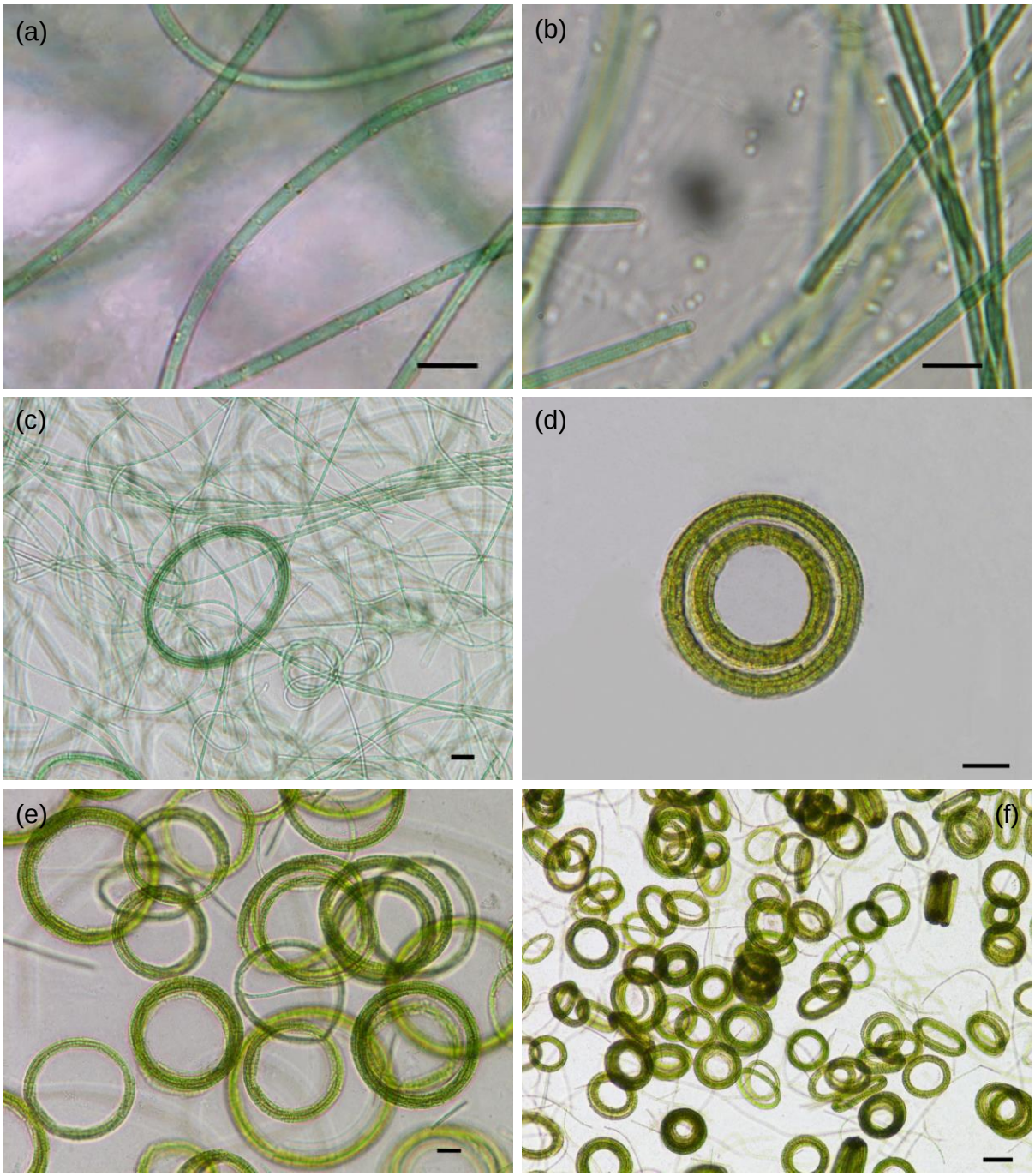

Figure 10. Light microscopy micrographs of *Sodalinema komarekii* (strain PMC 869.14) from Dziani Dzaha. a: trichomes with light refractive granules; b: apical cells rounded with a large granule (aerotope?); c: mixed straight and spirally coiled filaments within the same strain; d-f: trichomes spirally coiled. Scale bars: a, b = 10 µm; c, e = 20 µm; f = 50 µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

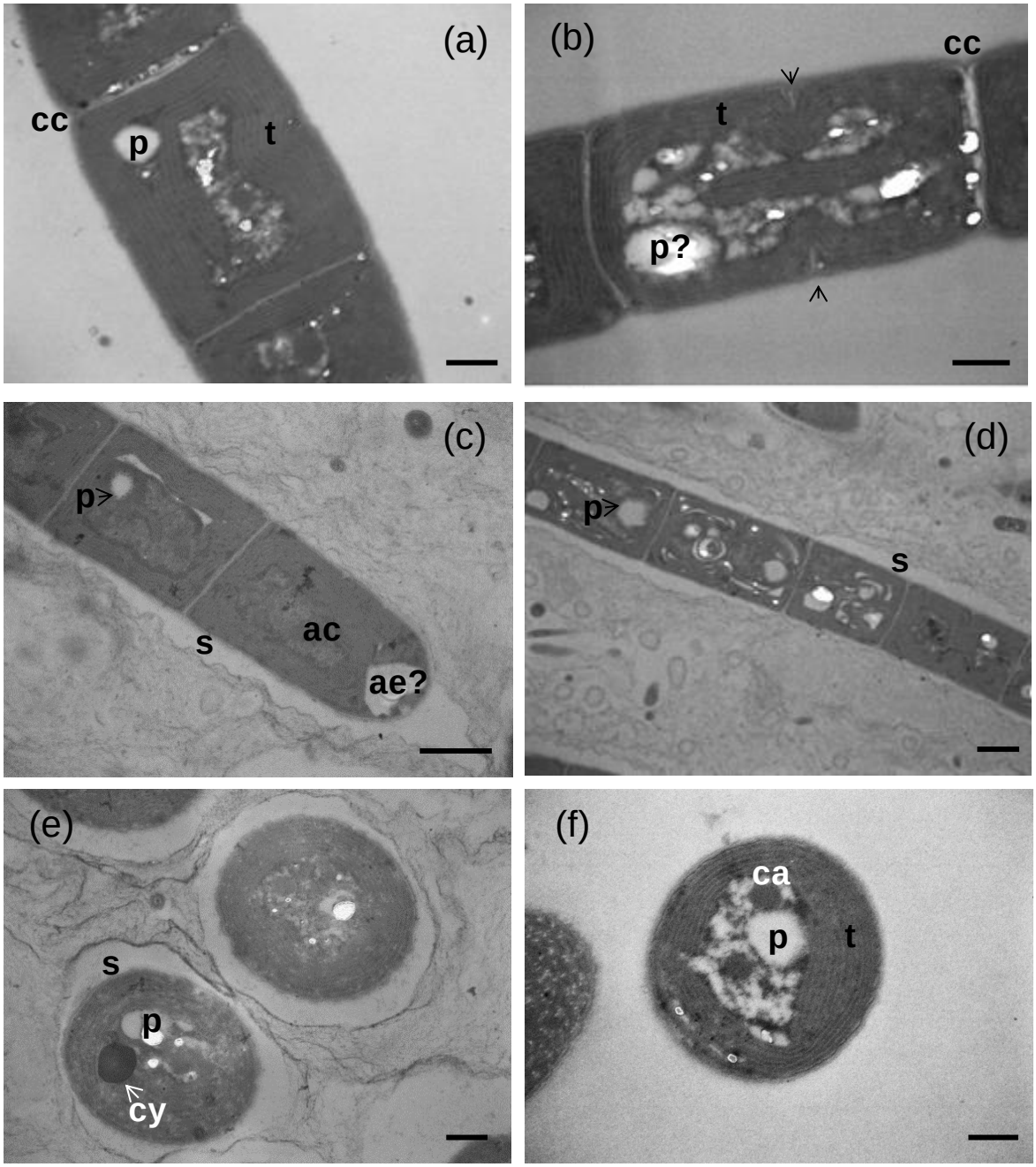

Figure 11. TEM micrographs of *Sodalinema komarekii* (strain PMC 869.14) from Dziani Dzaha. Longitudinal (a-d) and cross sections (e-f) of the trichome showing the ultrastructural details. Figure b also shows a potentially polyphosphate granule and a cell undergoing binary fission (arrows indicate cell constriction). Abbreviations: ac: apical cell, ca: carboxysome, cc: cross-wall constriction, cy: cyanophycin granule, p: polyphosphate granule, s: sheath, t: thylakoids. Scale bars: a, b, e, f = 500 nm; c, d = 1µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

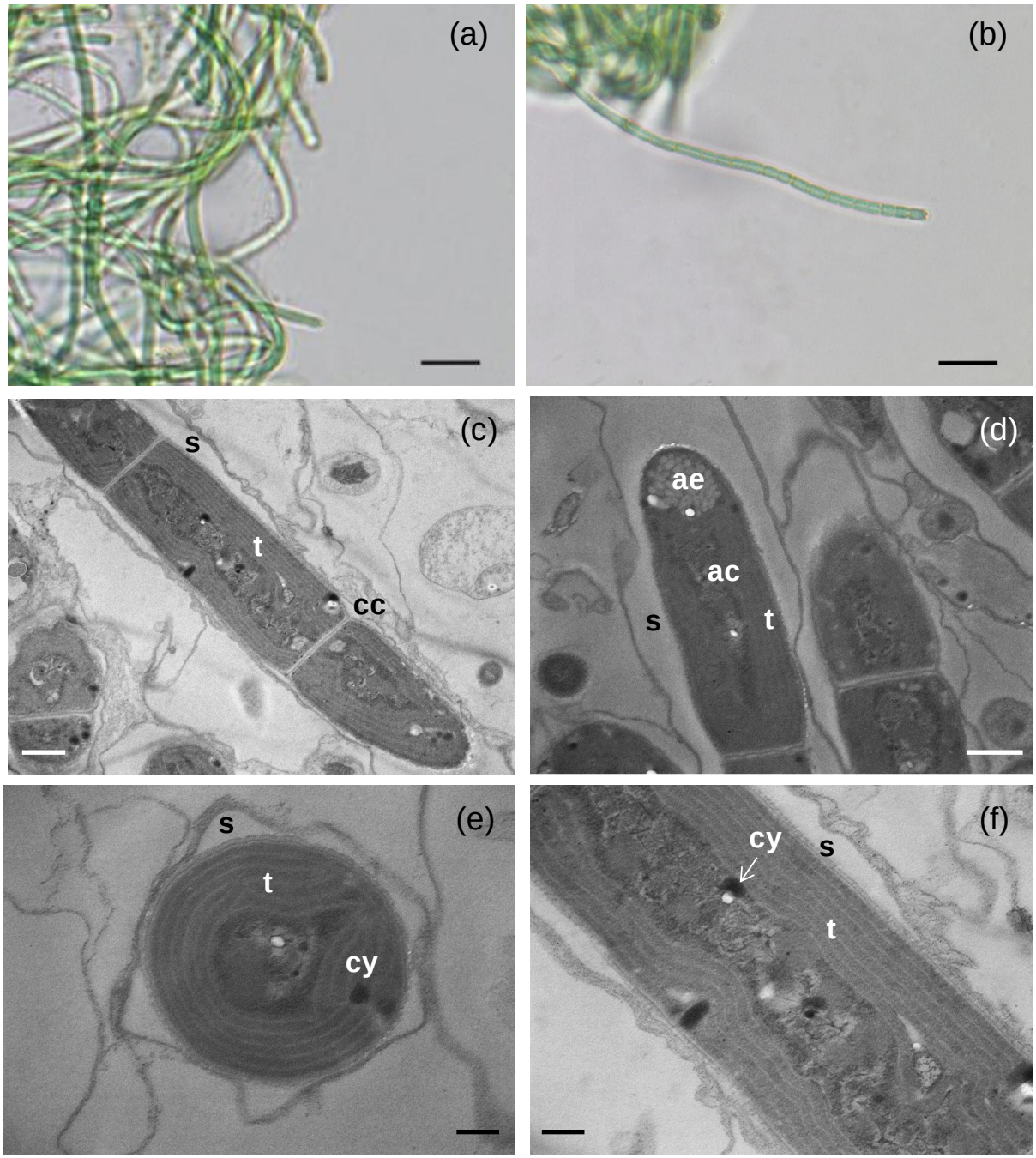

Figure 12. Light microscopy (a-b) and TEM (c-f) micrographs of *Sodaleptolyngbya stromatolitii* (strain PMC 867.14) from Dziani Dzaha. Entangled filaments and trichome with sheath (a); trichomes with refractive granule in the apical cell (b), longitudinal (c-d-f) and cross sections (e) of the trichome showing the ultrastructural details. Abbreviations: ac: apical cell, ae: aerotopes, cc: cross-wall constriction, cy: cyanophycin granule, s: sheath, t: thylakoids. Scale bars: a, b = 10 µm; c, d = 500 nm; e, f = 200 nm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

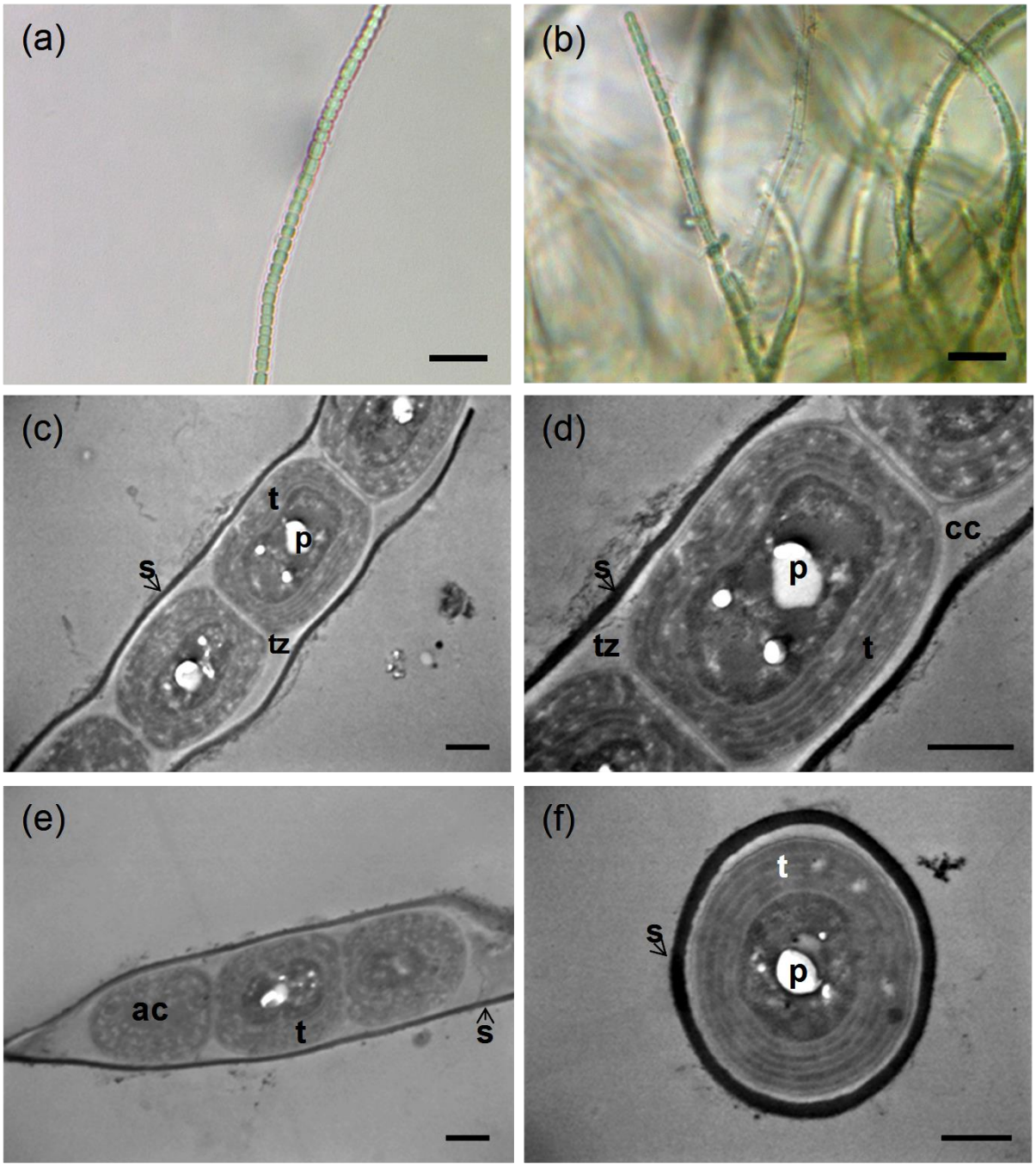

Figure 13. Light microscopy (a-b) and TEM (c-f) micrographs of *Haloleptolyngbya alcalis* (strain PMC 892.15) from Dziani Dzaha. Solitary filament (a), entangled filaments and trichome with sheath (b), longitudinal (c-e) and cross sections (f) of the trichome showing the ultrastructural details. Abbreviations: ac: apical cell, cc: cross-wall constriction, p: polyphosphate granule, s: sheath, t: thylakoids, tz: transparent zone. Scale bars: a, b = 10 µm, c, d, e, f = 500 nm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

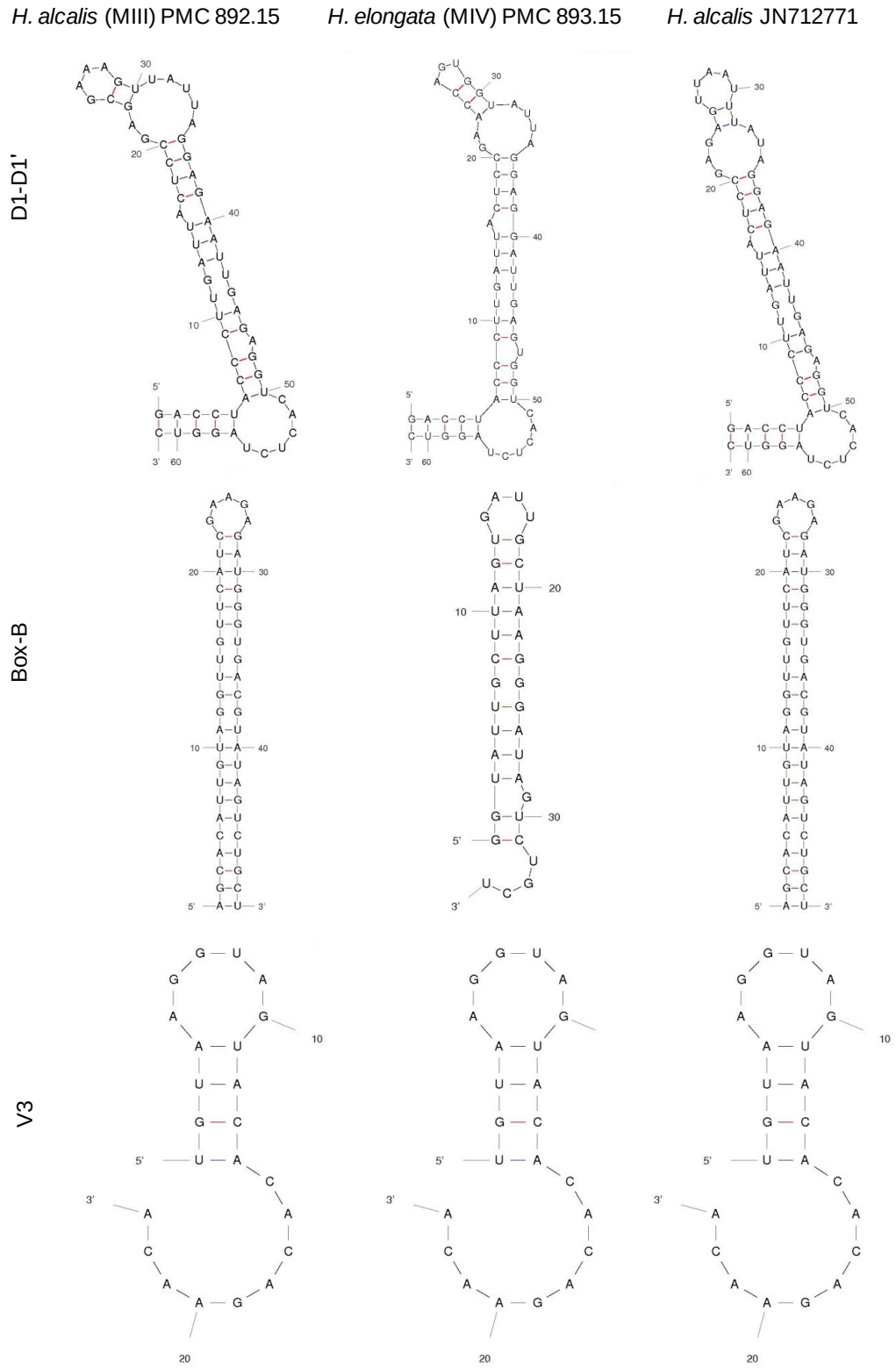

Figure 14. 16S-23S ITS secondary structures (D1-D1', Box-B and V3) of *Haloleptolyngbya alcalis* and *H. elongata* from Dziani Dzaha and *H. alcalis* from Dadheech *et al.* (2012b). M: morphotype.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

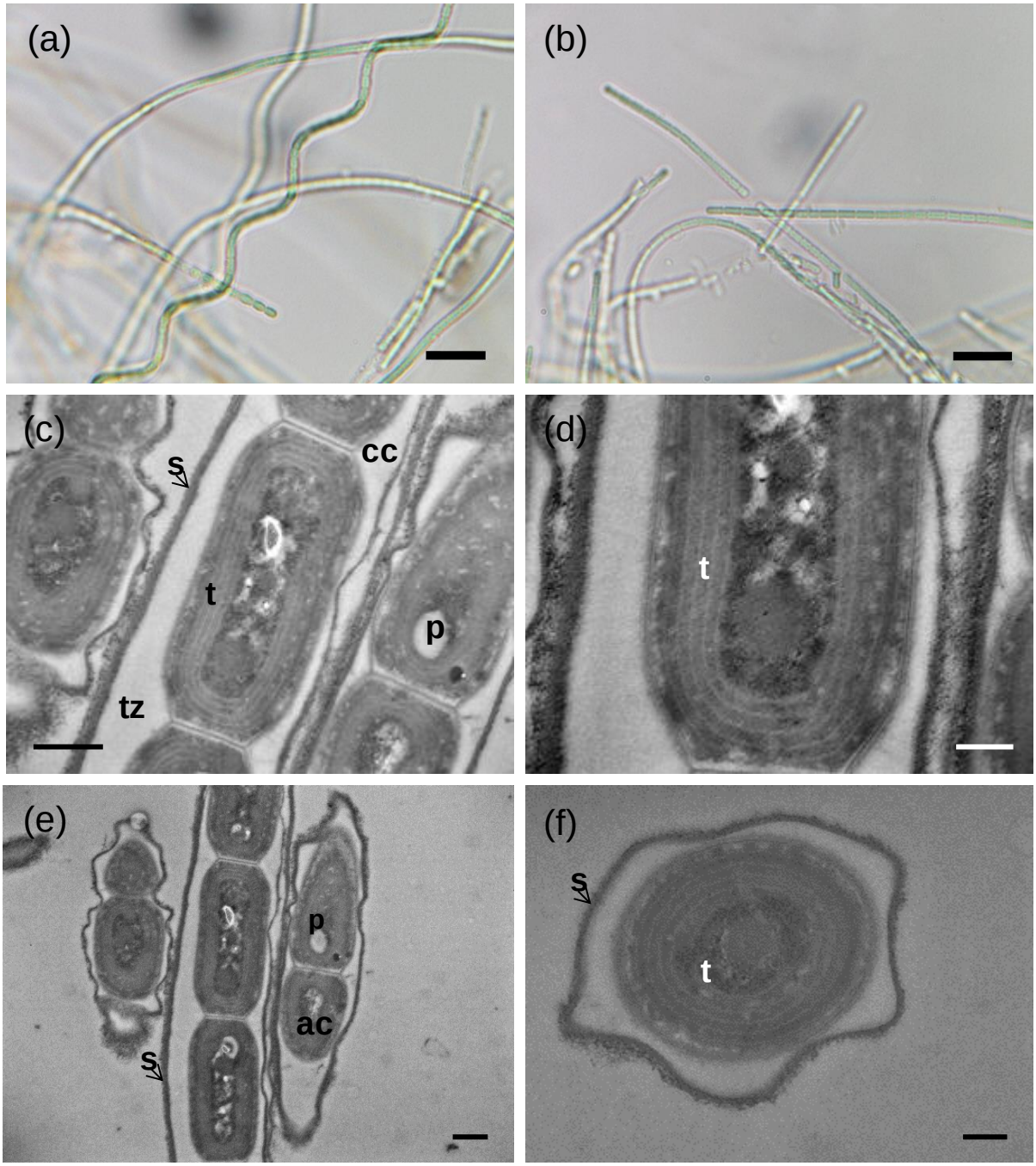

Figure 15. Light microscopy (a-b) and TEM (c-f) micrographs of *Haloleptolyngbya elongata* (strain PMC 895.15) from Dziani Dzaha. Flexuous and undulated filaments (a), trichome with sheath (b), longitudinal (c-e) and cross sections (f) of the trichome showing the ultrastructural details. Abbreviations: ac: apical cell, cc: cross-wall constriction, p: polyphosphate granule, s: sheath, t: thylakoids, tz: transparent zone. Scale bars: a, b = 10 µm; c, e = 500 nm; d, f = 200 nm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

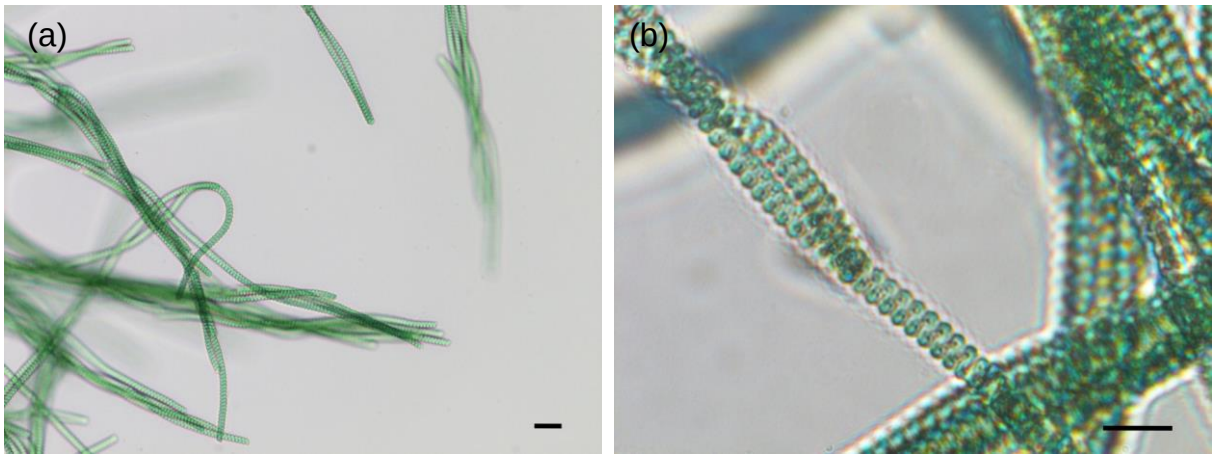

Figure 16. Light microscopy micrographs of *Spirulina subsalsa* (strain PMC 857.14) from Dziani Dzaha. a: Filaments entangled, b: trichome regularly densely screw-like coiled. Scale bars: a = 20 µm, b =10 µm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

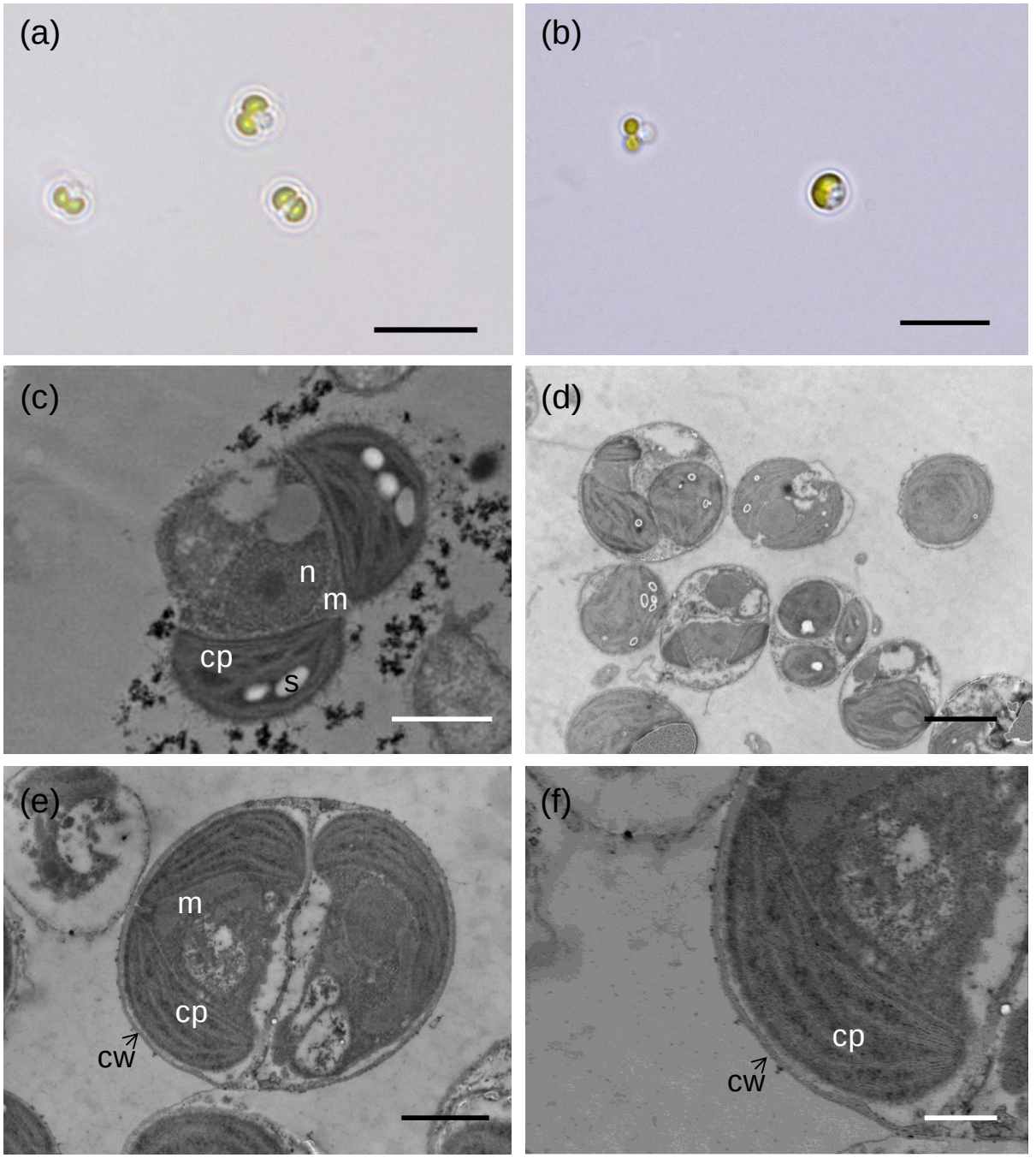

Figure 17. Light microscopy (a-b) and TEM micrographs (c-f) of *Picocystis salinarum* (a, b, d, e, f: strain ALCP 144.1; c: environmental sample) from Dziani Dzaha. a: trilobate cells; b: trilobate and slightly bilobed cells; c: trilobate cell with starch grains and chloroplasts in the lateral lobes. The nucleus and the mitochondrion are located in the third lobe; d: autospores and daughter cells within the mother cell wall; e: daughter cells within the mother cell wall; f: detail of the figure e showing the cell-wall and the cup-shaped chloroplast. Abbreviations: cp: chloroplast; cw: cell-wall; m: mitochondrion; n: nucleus; s: starch grain. Scale bars: a-b = 10 µm; c, e = 1 µm; d = 2 µm; f = 200 nm.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

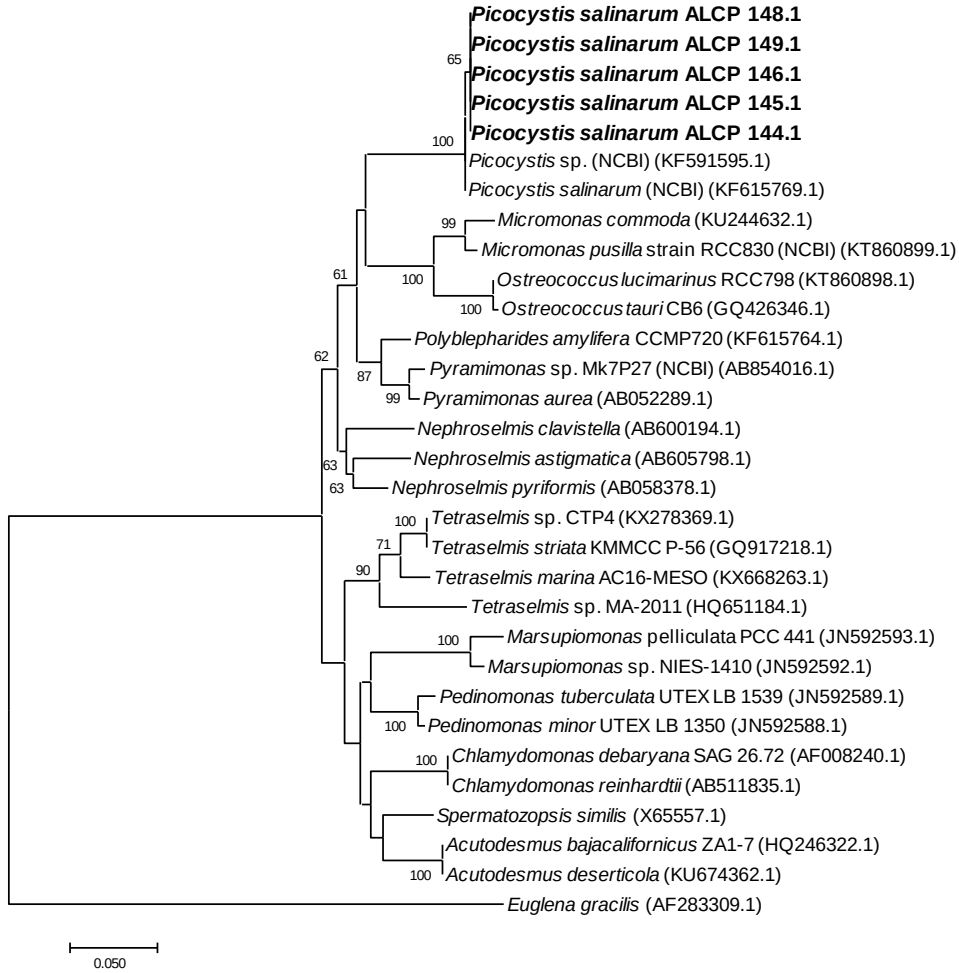

Figure 18. Neighbor joining phylogenetic tree based on 18S rRNA gene sequences of the prasinophyte *Picocystis salinarum*. Numbers above branches indicate bootstrap support (>50 %) from 1000 replicates. *Picocystis* strains from Dziani Dzaha strains are in bold.

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Table 1. List of cyanobacteria and microalgae strains isolated from Dziani Dzaha and their corresponding strain numbers, habitat and sampling dates. PMC: Paris Museum Collection. ALCP: Algothèque Laboratoire Cryptogamie Paris. DZ10-10: October 2010. DZ14-04: April 2014.

Taxa	Strain number	Habitat	Sampling date
Arthrospira fusiformis	PMC 737.11; 738.11	Water column	DZ10-10
Arthrospira fusiformis	PMC 851.14 to 853.14	Stromatolites	DZ14-04
Arthrospira fusiformis	PMC 894.15; 912.15 ; 917.15	Water column	DZ14-04
Desertifilum dzianense sp. nov.	PMC 871.14 to 876.14; 890.15; 901.15; 902.15; 905.15 ; 906.15; 909.15	Stromatolites	DZ14-04
Sodalinema komarekii gen. nov., sp. nov. (Morphotype I)	PMC 741.11 to 746.11	Water column	DZ10-10
Sodalinema komarekii gen. nov., sp. nov. (Morphotype I)	PMC 861.14 to 865.14 ; 869.14	Stromatolites	DZ14-04
Sodalinema komarekii gen. nov., sp. nov. (Morphotype I)	PMC 886.15 to 889.15; 898.15; 899.15; 907.15	Water column	DZ14-04
Sodaleptolyngbya stromatolitii gen. nov., sp. nov. (Morphotype II)	PMC 866.14; 867.14	Stromatolites	DZ14-04
Haloleptolyngbya alcalis (Morphotype III)	PMC 891.15, 892.15	Water column	DZ14-04
Haloleptolyngbya elongata sp. nov. (Morphotype IV)	PMC 893.15; 895.15; 900.15; 903.15; 904.15	Water column	DZ14-04
Spirulina subsalsa	PMC 857.14, 858.14	Stromatolites	DZ14-04
Picocystis salinarum	ALCP 144.1 to 146.1; 148.1; 149.1	Water column	DZ14-04

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Table 2. Comparison of the morphological features of *Desertifilum dzianense* sp. nov. with other *Desertifilum* species.

Characteristics	*Desertifilum dzianense*	*Desertifilum tharense*	*Desertifilum fontinale*	*Desertifilum salkalinema*
Aerotopes	Absent	Present	Absent	Absent
Filament towards the end	Attenuated at the ends	Attenuated at the ends	Attenuated at the ends	Attenuated or not attenuated at the ends,
Filament constriction	Unconstricted or slightly constricted at cross walls	Unconstricted or slightly constricted at cross walls	Unconstricted or slightly constricted at cross walls	Unconstricted or slightly constricted at cross walls
Filament type	Straight or slightly wavy/solitary or entangled	Straight or slightly wavy/solitary or entangled	Straight or slightly wavy/solitary or entangled	Straight or slightly wavy/ entangled
Sheath	Thin, colourless, attached to trichome	Thin, colourless, attached to trichome (agglutinated)	Thin and colourless	Thin, colourless, attached to trichome
Filament color	Blue-green	-	Blue-green	-
Motility	Present	Present	Present	Present
Necridic cells	Absent	-	Present	-
Thylakoids arrangement	Parietal	Parietal	Parietal	Parietal
Apical cell	Conical shaped with rounded apices, slightly hooked or bent, sometimes with extrusions	Conical or variable	Conical shaped with rounded apices, sometimes with extrusions	Slightly elongated
Cell shape	Cylindrical, mainly longer than wide (rarely isodiametric)	Cylindrical, isodiametric, longer than wide	Cylindrical, isodiametric, shorter than wide	Cylindrical, longer than wide
Cell width (µm)	2.4 – 3.4	2.0–3.7	4-7	2.08 (±0.8)
Cell length (µm)	3.4 – 5.9	-	-	5.14 (±0.5)
Habitat	Stromatolites (Dziani Dzaha lake, Mayotte)	Desert crust or arid	Free floating mats in a warm spring near Lake Bogoria, Kenya	Alkaline cultivated pool
Reference	This study	Dadheech *et al.* (2012a)	Dadheech *et al.* (2014)	Cai *et al.* (2017)

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018

Table 3. Comparison of the *Leptolyngbya*-like strains (morphotypes I-IV) isolated from Dziani Dzaha (Mayotte).

Characteristics	**Morphotype I**	**Morphotype II**	**Morphotype III**	**Morphotype IV**
	Sodalinema komarekii gen. nov., sp. nov.	*Sodaleptolyngbya stromatolitii* gen. nov., sp. nov.	*Haloleptolyngbya alcalis*	*Haloleptolyngbya elongata* sp. nov.
Filament shape	Nearly straight, flexuous, forming circular bundles	Nearly straight, slightly flexuous	Flexuous, sometimes wavy	Straight, slightly arcuated, flexuous, sometimes undulated
Filament towards the end	Very slightly attenuated	Not attenuated	Not attenuated	Not attenuated
Constriction at cross-wall	Slightly constricted	Slightly constricted	Very constricted	Very constricted
Sheath	Present	Present	Present	Present
Transparent zone	Absent	Absent	Present	Present
Trichome motility	Present	Present	Absent	Absent
Thylakoids arrangement	Parietal	Parietal	Parietal	Parietal
Cell shape	Cylindrical, longer than wide	Cylindrical, longer than wide	Barrel-shaped, isodiametric, shorter or longer than wide	Cylindrical, longer than wide (up to 3 times)
Cell width (µm)	2.4-3	1.5-2	1.4-1.9	0.9-1.5
Apical cell type	Rounded, hemispherical	Rounded	Conical-rounded	Conical-rounded
Aerotopes	Refractive granule in the apical cell (?)	Present (numerous aerotopes in the apical cell)	Absent	Absent
Particular features	A large refractive granule on either or both sides of the cross-walls and in the apical cell	A large refractive granule in the apical cell (numerous aerotopes)	-	-
Habitat	Water column and stromatolites	Stromatolites	Water column	Water column

Downloaded from https://academic.oup.com/femsec/advance-article-abstract/doi/10.1093/femsec/fiy108/5033403
by Museum National d'Histoire Naturelle user
on 06 June 2018