



Taxonomic and Ecological Observations on Some Algal and Cyanobacterial Morphospecies New for or Rarely Recorded in Either Egypt or Africa

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THE UNDERSTANDING of the diversity and spatial distribution of cyanoprokaryotes and algae in Egypt is challenging because this is still an understudied topic. To address this knowledge gap, we discuss morphotaxonomic features and ecological preferences for ten cyanobacterial and algal morphospecies from diverse Egyptian biotopes. Morphospecies were studied and identified using state-of-the-art and fine-grained taxonomy based on light and scanning electron microscope observations. Of these taxa, the cyanoprokaryotes *Lemmermanniella uliginosa* and *Scytonema myochrous*, the freshwater diatoms *Cyclotella meduanae*, *Cavinula lapidosa*, and *Craticula subminuscula*, the unicellular chrysophyte *Mallomonas crassisquama*, and the worldwide rarely-recorded zygnematalean streptophyte *Hallasia* cf. *reticulata* are designated as new records for Egypt. Moreover, the latter and *L. uliginosa* are as well first records for the whole African continent. Worthy of note, the freshwater diatoms *Cyclostephanos invisitatus*, *Encyonema neomesianum*, and *Gomphonema laticollum* have been rarely observed in previous Egyptian studies. Overall, most taxa identified tolerate increased nutrient concentrations (meso-eutrathentic and eutrathentic species), reflecting different human impacts on the biotopes they colonize. These newly recorded taxa are supposed to have been mostly overlooked in the previous Egyptian studies due to their relatively isolated habitats, small size, or complex taxonomic and nomenclatural history. More interesting algal and cyanobacterial taxa are still hidden and not yet discovered in Egypt, particularly in the little-explored and isolated desert habitats, and further research, using integrated polyphasic approaches, is therefore indispensable to achieve a better estimate of the Egyptian cyanobacterial and algal diversity, and to set up efficient algae-based assessment systems tailored for the Egyptian habitats.

Keywords: Africa, Algae, Cyanoprokaryotes, Egypt, Diatoms, Modern morphotaxonomy, New records.

Introduction

In Egypt, there has been a plethora of floristic and taxonomic studies conducted to explore and characterize the diverse freshwater cyanoprokaryotic and algal assemblages, including

diatoms, from different biotopes across the country (e.g., Foged, 1980; Shaaban, 1994; El-Awamri et al., 1996; Hamed, 2005, 2008; Shanab, 2006; Mansour et al., 2015; Khairy et al., 2017; El-Sheekh et al., 2018; Saber et al., 2018a; Kassem et al., 2020 and the references therein). Species identifications

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in the above-mentioned literature and other papers, have been primarily based on light microscopy-uncovered morphological traits, overlooking ultrastructural details which are crucial for precise species delineation, particularly in diatoms. Besides the interest in their spatio-temporal distribution and ecological niches, algal- and diatom-based environmental assessment (El-Naghy et al., 2006; El-Sheekh et al., 2010; Shaaban et al., 2012, 2015; Abd El-Karim, 2014; Wołowski et al., 2017; Yusuf et al., 2018) and paleoenvironmental reconstructions (e.g., Zalat, 2003) have also been pursued in some studies. Given that Egyptian phycological investigations are mostly restricted to the River-Nile basin, lakes, pools, and agricultural drainages, the little-explored and untouched desert ecosystems in the Western and Eastern Deserts still await first or more intensive polyphasic studies, i.e. investigations combining information from state-of-the-art morphotaxonomy, autecology, ultrastructure, gene-markers' phylogenetics, and ecophysiological adaptive bioorganic traits (Cantonati et al., 2020b). It is also worth noting that a complete and reliable inventory, including all taxa identified is not available so far, and hence more detailed studies are still necessary in this respect. This is underpinned by the bibliography on the diversity of non-marine algae of Africa published by Levanets & Resburg (2010), in which the northeastern region is poorly represented in the ca. 2100 publications assessed.

In 1994, Shaaban published a thorough list of 912 freshwater species and infraspecies of cyanoprokaryotes and algae, including the ecological status and distributional patterns, inhabiting ecologically diverse inland waters, within the framework of the assessment of the biological diversity of Egypt. As concerns cyanoprokaryotes, he reported 170 different taxa belonging to 37 genera, and also stressed the extinction of 32 species since the early work of El-Nayal in 1935. Eleven years later, Hamed (2005) published an updated inventory about blue-green algae/cyanobacteria inhabiting the different water biomes in Egypt. This list included 290 putatively-cosmopolitan taxa, belonging to 51 genera of 9 families and 4 orders. In 2008, Hamed compiled the diversity and distribution of blue-green algae, and also diatoms, in some Egyptian inland water biotopes with respect to conductivity, and recorded 353 different species and infraspecies. He emphasized the difficult segregation of blue-green algae into marine and freshwater species but provided a diatom-based ecological characterization

of the habitats studied. With respect to diatoms, the previous contributions of Shaaban (1994), Hamed (2008), Saleh (2009), and other studies are among the main reliable taxonomic sources available as an aid for the identification of Egyptian diatoms but the nomenclatural system applied is nowadays obsolete. Species of Chlorophyta are also widely distributed in the diverse Egyptian habitats for which Shaaban (1994) reported 361 species representing 61 genera and 31 families. Of these, members of the family Zygnemataceae were rarely documented and were restricted to the genera *Mougeotia*, *Spirogyra*, and *Zygnema*. Recently, Shaaban et al. (2015) also reported an interesting species of the rarely known genus *Zygnemopsis*.

More interestingly, some algal and diatom species, which were new records for Egypt or even new to science, have been described from different biomes during the last years in the frame of integrative studies, and more endemic and cryptic species are predicted to be discovered in the future, particularly in the isolated arid-land freshwater ecosystems with less human impacts (Cantonati et al. 2020b). Saber et al. (2017b & 2018b), for instance, discovered an interesting *Rhizoclonium* sp. and the new desmid *Euastrum elfarafraense*, respectively, in an agricultural ditch fed by the rheocrenic hypothermal freshwater spring "Ain El-Balad" in the El-Farafra Oasis, Western Desert of Egypt, based on unique character combinations of morphology, autecology and molecular phylogenetics. The recently published work of Wołowski et al. (2017) on euglenoids of the same oasis reported 20 species, of which the colorless *Peranema inflexum* and the three pigmented species *Euglena adhaerens*, *Phacus crassus*, and *Ph. cristatus* were new records for Egypt. As regards diatoms, curved forms of *Aulacoseira ambigua* f. *japonica* were observed in the River Nile basin for the first time in Egypt, and this observation also represented the second record for the whole African continent (Janse van Vuuren et al., 2018). As part of the ongoing PhyBiO project on the diversity and distribution of the Egyptian algal flora, the new epilithic amphoroid diatom species *Seminavis aegyptiaca* has also been discovered from the Damietta Branch estuary of the River Nile (Saber et al., 2020).

To which extent is the Egyptian inland-waters' algal flora only a taxonomic artifact, and how many interesting and rarely observed species does it still harbor? The principal aim of this study was to contribute to a response to this important question,

and also to broaden and improve our knowledge on the Egyptian algal diversity. Morphotaxonomic diagnostic features, autecological preferences, and biogeographical data of ten rarely-observed and interesting cyanobacterial, diatom, and other-algae morphospecies were studied applying up-to-date taxonomic systems, light microscopy (LM), and scanning electron microscopy (SEM) approaches.

Materials and Methods

Sampling sites

During our recent surveys of the Egyptian freshwater algal flora, also in the frame of the ongoing PhyBiO project, cyanobacterial and algal specimens, on which this study is based, were sampled from three different ecosystems. All diatom (epilithon) specimens were gathered on August 25th 2016 from three sampling localities located in the northern part of the Damietta Branch of the River Nile (Fig. 1A–B). Sampling sites are as follows: Site 1 (31° 24' 0.9" N, 31° 45' 27" E) ca. 5km, Site 2 (31° 22' 57.4" N, 31° 44' 21.8" E) ca. 10km, and Site 3 (31° 20' 23.3" N, 31° 42' 44.5" E) ca. 15km upstream from the Faraskur Dam Barrage. The Damietta branch is one of the two branches of the River Nile, splitting North of Cairo and expanding to the East over a distance exceeding 240km until reaching the Mediterranean Sea. It has an average width of approximately 280m and a depth ranging between 12 and 20m. It is dammed about 20km inland of the river mouth by an engineered dam known as Faraskur Dam Barrage, forming the estuary. Along its main basin, the Damietta Branch serves as a pivotal water artery for a wide range of domestic, industrial, and agricultural, activities (Saber et al., 2020). The cyanoprokaryote *Lemmermanniella uliginosa* (Synechococcales, Cyanobacteria) was sampled on October 14th 2016 from metaphytic materials in an inland, mineral, ambient spring known as "Ain Al-Molouk" (29° 11' 2.7" N, 25° 33' 9.5" E) in the Siwa Oasis (Western Desert, Egypt) at an elevation of -7.0±9.1m b.s.l. The main springhead is surrounded by concrete walls and fully exposed to the sunlight (Fig. 1C–D). This spring is used for agricultural aims in this harsh desert habitat. The Siwa Oasis has a total area of about 800 km² and is characterized by a hot hyper-arid desert climate. It is also one of the smallest oases in the Western Desert, and lies at approx. 10–17m below the sea level (El-Sabbagh et al., 2017). Groundwater, derived from the Fissured Complex Carbonate and the Nubian Sandstone Aquifer Systems, is the only available water resource in this Saharan ecosystem.

The last sampling site (27° 3' 08.9" N, 33° 16' 54.4" E, ca. 737m a.s.l), where the heterocytous false-branching filamentous species *Scytonema myochrous* (Nostocales, Cyanobacteria), the unicellular chrysophyte *Mallomonas crassisquama* (Synurales, Ochrophyta), and the worldwide rarely known green filamentous species *Hallasia* cf. *reticulata* (Zygnematales, Streptophyta) were found, is located in a hyper-arid valley known as "Wadi Al-Naq'at" in the Eastern Desert of Egypt (Fig. 1E). This valley was visited on February 1st 2018. The first species was collected as a hygropetric, pseudoaerial mass growth with leathery and dark-brownish to black appearance on a dripping limestone rockwall (Fig. 1F), while the last two species were sampled from the periphytic algal materials growing on the ground beneath these rocks.



Fig. 1 (A–F). Landscape views of the sampling biotopes considered in the present paper [A: The main basin of the northern part of the Damietta Branch of the Nile, B: Epilithon where the diatom samples were collected from the Damietta Branch, C: The inland mineral ambient-temperature spring "Ain Al-Molouk" in the Siwa Oasis, D: Close-up view on Ain Al-Molouk springhead depicting algal and cyanobacterial assemblages, including *Lemmermanniella uliginosa*, E: Part of the hyper-arid valley "Wadi Al-Naq'at" at the Eastern Desert of Egypt, F: Hygropetric, pseudoaerial mass growth of the heterocytous false-branching filamentous species *Scytonema myochrous* with dark-brownish to black appearance on a dripping limestone rockwall in Wadi Al-Naq'at]

Sample processing, and taxa identification

All the specimens were collected following the European standard methods for sampling phytobenthos in running waters (EN 15708, 2009). With regard to diatoms, small subsamples were cleaned by adding hot 37% hydrogen peroxide (H₂O₂) and hydrochloric acid (HCl) to get rid of organic matter and carbonate particles, and thereafter rinsed three times with deionized water. The material was diluted with distilled water to avoid excessive diatom-valve concentrations. Cleaned diatom frustules were mounted in Naphrax[®], a synthetic mounting medium with a high refractive index of 1.74. The permanent slides were analyzed using the oil immersion objective at 1000x magnification. Light microscopy (LM) observations were conducted using a Zeiss Axioskop 2 microscope (Zeiss, Jena, Germany) equipped with phase-contrast, and an Axiocam digital camera at the MUSE – Museo delle Scienze, Limnology & Phycology Section, Trento, Italy, and also a BEL[®] photonics biological light microscope (BEL[®] Engineering, Monza, Italy) at the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt, where micrographs were taken with a Canon Powershot G12 digital camera. For the biometric data provided, measurements on at least 25 different specimens representative of the size-diminution series were conducted to obtain ranges and averages of the morphological and ultrastructural features. For scanning electron microscopy (SEM) analysis, a few drops of the cleaned diatom suspensions were mounted and air-dried onto small round aluminum stubs and sputtered with chromium (Cr). SEM observations were performed using Sigma[®] 300 VP electron microscope (Carl Zeiss) at 5kV at the Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia. The slides and prepared materials are deposited at the Phycology Unit (No. 341), the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. The terminology for valve morphology is primarily based on Round et al. (1990).

For identification of the different taxa in this study, the following taxonomic references were used: Komárek & Komáková-Legnerová (2007) and Komárek (2013) for Cyanobacteria; Krammer & Lange-Bertalot (1991), Krammer (1997), Houk et al. (2010 & 2014), Levkov et al. (2016), and Lange-Bertalot et al. (2017) for Bacillariophyta; Kristiansen & Preisig

(2007) for Chrysophyta; Kadlubowska (1984) for Zygnematales. The worldwide geographical distribution maps of *Lemmermanniella uliginosa* and *Hallasia* cf. *reticulata* were constructed using MapChart.net, available from <https://mapchart.net/world.html>.

Hydrochemical characterization

Detailed hydrochemical characteristics of the sampling sites, including major ions, nutrients, trace elements, and metals, were obtained following standard procedures and methods adopted by Chapman & Pratt (1978) and Clesceri et al. (2000). Major cations (Na⁺, K⁺, Ca²⁺, and Mg²⁺), anions (Cl⁻, HCO₃⁻, CO₃²⁻, and SO₄²⁻), and trace elements and metals were determined using ionic chromatography (ICS 1500 Dionex Corp.). Nutrients (NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, TP, and SRP) were measured by molecular absorption spectrometry. Silicates (SiO₂) were analyzed by the molybdosilicate method. *In situ* water temperature, pH, ion conductivity, and total dissolved solids (TDS) were measured with a calibrated HANNA HI 991301 meter. Dissolved Oxygen (DO) was as well measured in-field with a calibrated Lutron[®] YK-22DO (Lutron Electronic Enterprise Co., Ltd., Taiwan) meter.

Results and Discussion

In the present study, ten different cyanobacterial, diatom, and other-algae morphotypes were identified and discussed from the standpoints of state-of-the-art morphotaxonomy and of ecological characterization. Out of these ten, the cyanoprokaryotes *Lemmermanniella uliginosa* and *Scytonema myochrous*, the diatoms *Cyclotella meduanae*, *Cavinula lapidosa*, and *Craticula subminuscula*, the chrysophyte *Mallomonas crassisquama*, and the filamentous streptophyte *Hallasia* cf. *reticulata*, turned out to be new records for the Egyptian algal inventory. *Hallasia* cf. *reticulata* and *Lemmermanniella uliginosa* represent also the first records for the whole African continent. The freshwater diatoms *Cyclostephanos invisitatus*, *Encyonema neomesianum*, and *Gomphonema laticollum* have been rarely documented in the previous Egyptian studies. Detailed descriptions, ecological preferences, and biogeography of all the morphospecies recorded are given in the following. The hydrochemical characteristics of the habitats studied are provided in Table 1.

TABLE 1. Hydrochemical variables of the sampling sites where the cyanobacterial and algal morphospecies were collected in the present study

Parameter	Unit	Damietta Branch (The Nile River)*	The ambient mineral spring "Ain Al-Molouk" (the Siwa Oasis)	The hyper-arid valley "Wadi Al-Naq'at" (the Eastern Desert of Egypt)
Water temperature	°C	33.77	22.4	20.4
pH		7.96	6.98	8.81
Dissolved Oxygen (DO)	mg.L ⁻¹	5.7	6.6	–
Conductivity (EC)	µS.cm ⁻¹	449	2610	550
Total Dissolved Solids (TDS)	mg.L ⁻¹	226.7	1210.0	352.0
Sodium (Na ⁺)	mg.L ⁻¹	34.1	405.5	81.4
Potassium (K ⁺)	mg.L ⁻¹	8.0	18.3	13.0
Calcium (Ca ²⁺)	mg.L ⁻¹	37.2	96.4	25.5
Magnesium (Mg ²⁺)	mg.L ⁻¹	10.6	38.7	4.3
Chloride (Cl ⁻)	mg.L ⁻¹	22.0	654.0	121.4
Carbonate (CO ₃ ²⁻)	mg.L ⁻¹	0.0	0.0	0.0
Bicarbonate (HCO ₃ ⁻)	mg.L ⁻¹	200.5	218.0	112.9
Sulphate (SO ₄ ²⁻)	mg.L ⁻¹	27.7	196.2	10.8
Soluble Reactive Phosphate (SRP)	µg.L ⁻¹	488	20	40
Total phosphorus (TP)	µg.L ⁻¹	–	26	95
Nitrite (NO ₂ ⁻)	µg.L ⁻¹	43	6	0
Nitrate (NO ₃ ⁻)	µg.L ⁻¹	1567	164	43
Ammonium (NH ₄ ⁺)	µg.L ⁻¹	87	0	15
Silicate (as SiO ₂)	mg.L ⁻¹	9.2	1.8	0.7
Barium (Ba)	µg.L ⁻¹	37	6	–
Chromium (Cr)	µg.L ⁻¹	20	1	–
Cadmium (Cd)	µg.L ⁻¹	2.1	1.0	–
Aluminium (Al)	µg.L ⁻¹	10	6	–
Copper (Cu)	µg.L ⁻¹	5	9	6
Iron (Fe)	µg.L ⁻¹	14	8	100
Lead (Pb)	µg.L ⁻¹	26	3	–
Manganese (Mn)	µg.L ⁻¹	9	5	4
Nickel (Ni)	µg.L ⁻¹	1	25	–
Zink (Zn)	µg.L ⁻¹	1.5	0.1	10

*Weighted average values of the hydrochemical variables of the three sampling sites in the Damietta Branch of the Nile River. "–" means not detected.

1. *Lemmermanniella uliginosa* Komárek & Komárková-Legnerová **Fig. 2A–F**

Reference: Komárek & Komárková-Legnerová (2007) (p. 102, fig. 37: a–i)

Description: Colonies benthic, metaphytic, spherical (Fig. 2A–C), ± irregularly oval in older colonies (Fig. 2D–E), surrounded by distinct colourless, lamellated mucilage, 50–110 µm in diameter. Cells pale bluish-green, elongated-cylindrical "rod-like" with rounded ends, without aerotopes, irregularly arranged tangentially to

the colony surface, ± densely distributed in the periphery of smaller colonies, contents finely granular, 5.5–6.0 (–7.0) × 2.5–3.0 (–3.5) µm. Cell division by transverse binary fission perpendicular to the longitudinal axis. Reproduction by the disintegration of colonies, or by separating small spherical clusters of cells from the mother colonies.

Distribution in Egypt: This is the first record of this species in Egypt, where it was found in the inland, mineral, ambient-temperature spring

“Ain Al-Molouk” in the Siwa Oasis, the Western Desert of Egypt.

General distribution and ecology: As known so far, this morphospecies has a limited geographical distribution, having been recorded only in five countries: Belize (Komárek & Komárková-Legnerová, 2007), Australia and New Zealand (Bostock & Holland, 2010; McGregor, 2013), Peru (Mendoza-Carbajal, 2020), and the United States (Mareš, 2006). It therefore represents a new record for the African continent (Fig. 2F).

In Egypt, it was highly abundant in Ain Al-Molouk ambient spring among other metaphytic cyanobacteria such as *Chroococcus minutus* and *Leptolyngbya* sp. This groundwater-dependent biotope was mainly characterized by: - constant water temperature (°C): 22.4; - neutral pH: 6.98; - high electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$): 2610; - Na^+ and Cl^- were the major ions: 405.48 and 654.03 $\text{mg}\cdot\text{l}^{-1}$, respectively. Nitrates were low and TP presented moderate levels (164 and 26.0 $\mu\text{g}\cdot\text{L}^{-1}$, respectively), reflecting the oligo-mesotrophic status of this ecosystem (Table 1).

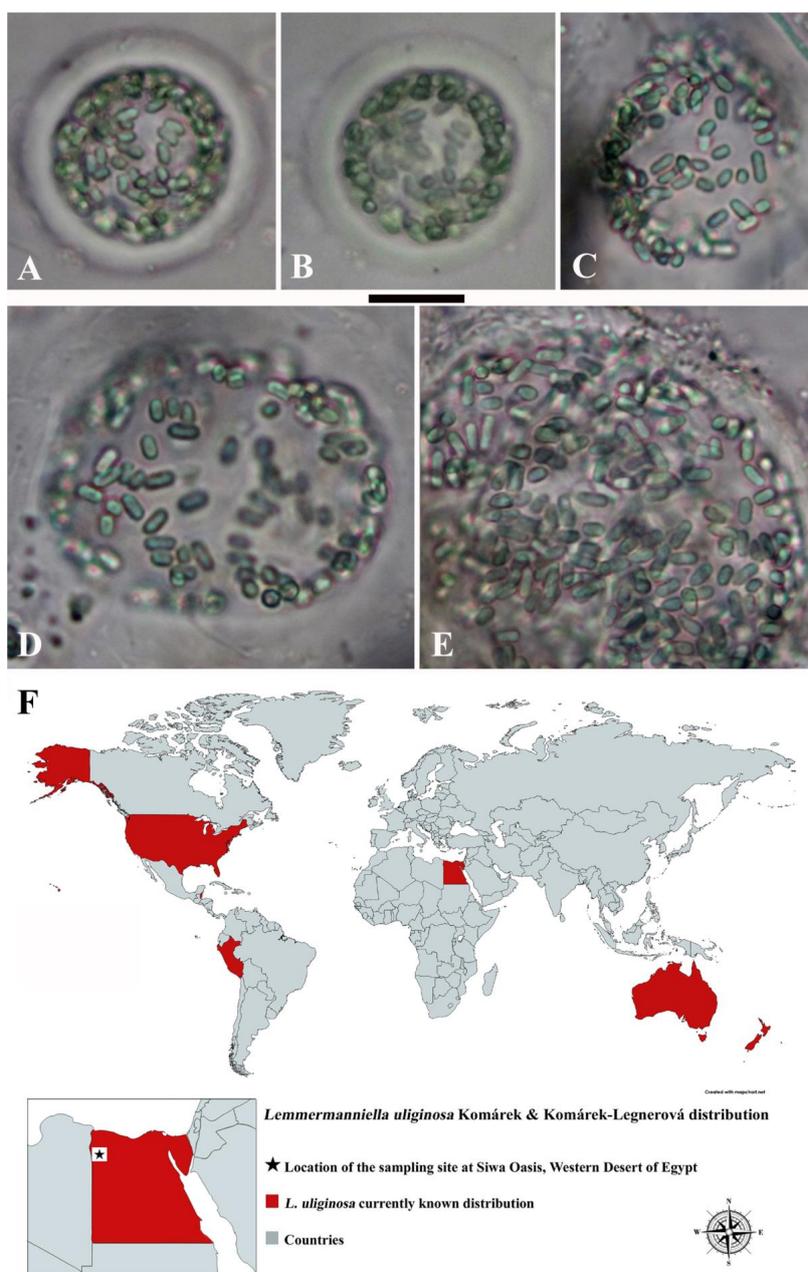


Fig. 2 (A–F). *Lemmermanniella uliginosa*; A–C: Young colonies with distinct colourless mucilage, D–E: Old colonies, F: *L. uliginosa* currently-known worldwide distribution [Scale bar= 20 μm].

Comments: Our specimens highly resemble the holotype population described by Komárek & Komárková-Legnerová (2007) from Belize. Ecologically, they reported it as benthic, metaphytic species growing in mats amongst other cyanoprokaryotes in tropical Central American alkaline marshes, and with lower abundance in those with higher conductivity. McGregor (2013) also found it as a benthic freshwater cyanobacterium in North-Eastern Australia.

2. *Scytonema myochrous* (Dillwyn) C.A. gardh ex Bornet et Flahault Fig. 3A–K

Reference: Komárek (2013) (p. 119, fig. 99: a–h; fig. 100: a–e)

Description: Thallus leathery, crusty, blackish-green to brownish-black (Fig. 3A). Filaments \pm entangled, 27.5–30 (–35) μ m wide, with usually common false branching; branches solitary (Fig. 3B–D) or in pairs (Fig. 3E), long, often narrower than main filaments. Sheaths dark yellow to yellowish-brown, with distinctly divergent layers mainly in old main filaments (Fig. 3E–K). Trichomes cylindrical, slightly constricted at cross-walls. Cells bluish-green to olive-green, cylindrical to quadratic or shorter than wide, 4.0–12.0 \times 7.0–10.0 μ m, end cells rounded (Fig. 3F). Heterocytes obliquely elliptic to rectangular-rounded, wider than vegetative cells (Fig. 3K), usually up to 15 μ m wide.

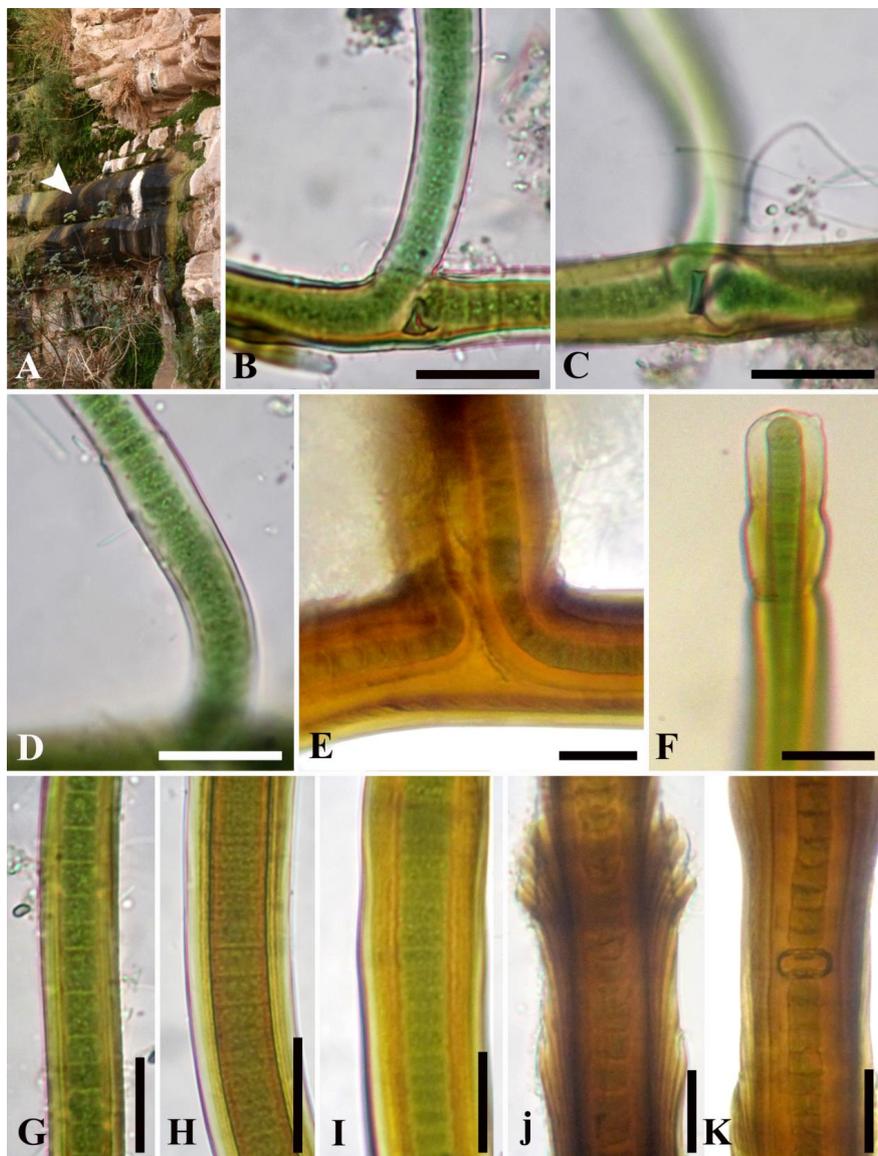


Fig. 3 (A–K). *Scytonema myochrous*; A: Dripping limestone bare rockwall covered by *S. myochrous* population, B–D: Solitary false-branching, E: Double false-branching, F–K: Details of the filaments showing dark-yellow to yellowish-brown sheaths with divergent layers, rounded end cell, and intercalary heterocyte [Scale bar=20 μ m]

Distribution in Egypt: This is the first record of the species for the Egyptian freshwater cyanoprokaryotes. It was found as hygropetric pseudoaerial mass growth on the dripping limestone rockwall in the hyper-arid valley “Wadi Al-Naq’at” in the Eastern Desert of Egypt. Environmental conditions of this biotope: - water temperature (°C): 20.4; - pH : alkaline (8.81); - conductivity ($\mu\text{S.cm}^{-1}$): 550; - major cations and anions: Na^+ , Cl^- , and HCO_3^- (81.43, 121.43, and 112.89 mg.l^{-1} , respectively); - SRP ($\mu\text{g.L}^{-1}$): 40; - TP ($\mu\text{g.L}^{-1}$): 95; - NO_3^- -N ($\mu\text{g.L}^{-1}$): 43.0; - NH_4^+ -N ($\mu\text{g.L}^{-1}$): 15.0; metals had average values reflecting the lithology of this desert habitat (Table 1).

General distribution and ecology: Cosmopolitan in tropical to northern regions. Moreover, this heterocytous species usually grows on wet soils, stones and rocks, often in dripping limestone areas and intensely wetted places, and less frequently in the littoral of lakes on submersed habitats (Komárek, 2013).

Comments: The morphotaxonomic features and ecological preferences of our specimens are identical to the diagnosis of populations illustrated in Komárek (2013). However, more integrative studies, including molecular phylogeny, should be pursued in the future to confirm the species identity. Komárek (2013) indeed stressed that this species is very polymorphic, and is likely to include several cryptic genotypes. He also highlighted that the tropical and subtropical populations from the southern hemisphere slightly differ from the type material. It is also worth indicating that the strain isolated from the Nile by Mohamed et al. (2006) is not identical to the holotype population and also to our specimens, particularly in the thallus structure, type of the branching, cell dimensions, and, above all, the autecology. On the contrary, Gesierich & Kofler (2010) described a *Scytonema myochrous* population from a tufa spring in the Austrian Alps, which strongly resembles our findings, not only in the morphology of the thalli and macroscopic aspect but also in the habitat colonized (hygropetric carbonate rock-wall associated with a small flowing spring). They even hypothesize that their population might be subject at times to desiccation, situation in which *S. myochrous* would have to obtain the nitrogen needed from the atmosphere.

3. *Cyclostephanos invisitatus* (M.H. Hohn & Hellermann) E.C. Theriot, Stoermer & Håkasson Fig. 4A–H

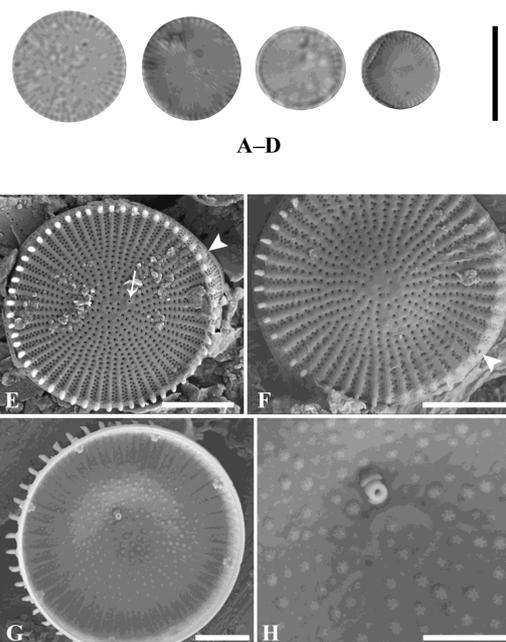


Fig. 4 (A–K). *Cyclostephanos invisitatus*; A–D: LM micrographs of valves showing size-diminution series, E: External valve view showing the marginal spines and subcentral (arrow) and marginal (arrowhead) fultoportulae openings, F: External valve view depicting the rimoportula opening (arrowhead). G: Internal view of valve showing details of central annulus and marginal fultoportulae, H: Close-up view on the subcentral fultoportula opening with two satellite pores [Scale bars= 10 μm (Figs A–D), 3 μm (Figs. E–F), 2 μm (Fig. G), 1 μm (Fig. H)]

Reference: Krammer & Lange-Bertalot (1991) (p. 63, fig. 674–3 :)

Description: Under the LM, valves discoid with a flat surface. Striae radial and delicate, only evident at the margins, 8.8–12.0 (–15) μm in diameter (Fig. 4A–D). With SEM, externally, the valve surface flat (Fig. 4E) or the central area might be slightly undulated (Fig. 4F). Striae radiate, finely punctate, bundled into fascicles, uniseriate in the central region, becoming biseriate towards the margin (Fig. 4E–F), 20–24 (–25) in 10 μm . Areolae 35–40 in 10 μm . Interstriae only discernible in the valve marginal area and extended onto the mantle (Fig. 4E–F). Elongated spines are present near the margin on every interfascicle. Fultoportulae located in the mantle with simple external pore-shaped tubes (Fig. 4E). Rimoportula simple, present on the mantle beneath one marginal spine (Fig. 4F). Internally,

areolae in the central region are occluded by domed cribra (Fig. 4H), while flat ones are present towards the margin and mantle (Fig. 4G). Interstriae are slightly elevated near the margins. Subcentral and marginal fultoportulae have two satellite pores (Fig. 4G–H). Rimoportula is small and sessile.

Distribution in Egypt: Rarely recorded in Egypt. Khairy et al. (2017), for instance, reported it from Lake Manzala. In the present study, it was rare in the epilithon of the Damietta Branch of the Nile River.

General distribution and ecology: Cosmopolitan, planktonic or benthic, alkaliphilic, eutraphentic diatom species usually present in aquatic habitats, like rivers and lakes, with a high conductivity (but Cl⁻ ions < 500mg.L⁻¹) and most often subjected to diverse human pressures (van Dam et al., 1994; Kiss et al., 2012; Cavalcante et al., 2013; Olszyński et al., 2019).

Comments: Our specimens resemble very well the populations studied by Kiss et al. (2012) from the large rivers in Hungary (i.e., 5–14µm in diameter, striae 14–22 in 10µm, and areolae density 20–40 in 10µm vs. 8.8–12.0 (–15)µm in diameter, striae 20–24 (–25) in 10µm, and areolae 35–40 in 10µm in the present study). Moreover, the Nile population have larger valve diameters and higher striae density than the type materials from North American rivers (i.e., 6–8µm in diameter, and 17 striae in 10µm based on our measurements from Fig. 19 in Theriot et al., 1987). We think this diatom species has been rarely observed and overlooked in the previous Egyptian studies due to the dependence of traditional taxonomic work upon light microscopy only. Being a widespread diatom species, it is very likely to be found in other freshwater ecotypes in Egypt in the future.

4. *Cyclotella meduanae* H. Germain Fig. 5A–J

Reference: Germain (1981) (p. 36, pl. 8, fig. 28; pl. 154, fig. 4: 4a)

Description: With LM, valves circular, 7.4–8.5µm in diameter, marginal striae radiate, 14–15 in 10µm (Fig. 5A–D). Under the SEM, externally, the valve central area is almost flat (Fig. 5E) to tangentially slightly undulated (Fig. 5F) with scattered spinulae that is evidently extended onto the valve face/mantle junction. Valve face fultoportula is clearly absent. Marginal fultoportulae 8–11, with short external tubes and

located on every 2nd to 3rd interstria (Fig. 5E–F). Rimoportula has a prominent external tube (Fig. 5E). Striae, in general ornate by small nodules, and each one has 8–9 rows of small, circular to ± irregular areolae (Fig. 5E–F). Internally, striae with smooth alveolar chambers. Fultoportulae have three satellite pores, and labiate process of the single rimoportula is obliquely oriented (Fig. 5G–H).

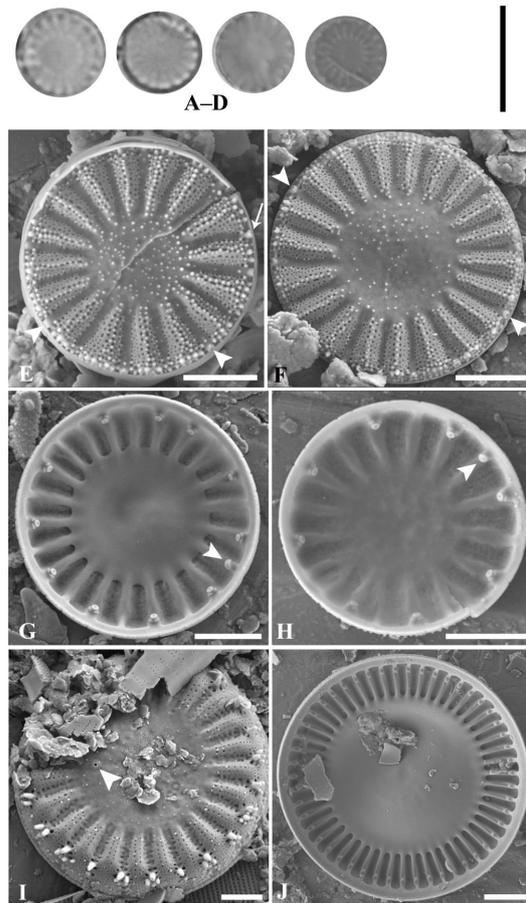


Fig. 5 (A–J). *Cyclotella meduanae*; A–D: LM micrographs of valves showing size-diminution series, E–F: External valve views depicting details of the central region, striae, rimoportula (arrow) and fultoportulae (arrowhead) openings, G–H: Internal valve views showing arrangement and details of rimoportula (arrowhead) and fultoportulae, I–J: Details of the internal and external valve views of the Nile *C. meneghiniana* for comparing with *C. meduanae* specimens. One central fultoportula is present (Fig. I; arrowhead) and internally marginal fultoportulae are located on all interstriae (Fig. J) [Scale bars= 10µm (Figs A–D), 4µm (Fig. J), 2µm (Figs E–I)]

Distribution in Egypt: This species is a new record for the Egyptian freshwater diatoms. It was dominant in the epilithon of the River Nile-Damietta Branch.

General distribution and ecology: Cosmopolitan, benthic or planktonic, alkaliphilic, freshwater diatom species, particularly in high-electrolyte content and nutrient-rich rivers, streams, and lakes (Håkansson, 2002; Kiss et al., 2010; Cavalcante et al., 2013).

Comments: The specimens described in our study highly resemble the protologue illustrated by Germain (1981). Taxonomically, *C. meduanae* might be confused with *C. meneghiniana*, particularly if species identification is carried out only with light microscopy. However, using SEM, there are some clear-cut differences to easily discriminate between the two species. *C. meneghiniana* usually has 1–4 central fulcra (Fig. 5I), while this taxonomic feature is totally absent in *C. meduanae*. Additionally, marginal fulcra in *C. meneghiniana* are internally located on all interstriae (Fig. 5J), but in *C. meduanae* they are distributed on every 2nd to 3rd interstria (Fig. 5G–H). The species *C. katiana*, recently discovered from a freshwater swamp in Colombia, also resembles *C. meduanae* in some characters, but the latter can be distinguished by the presence of elevated striae in relation to the valve surface (Sala & Ramírez-R, 2008). Besides its distinct structure of the central and marginal areas, *C. meduanae* also differs from *C. atomus* by lacking the distinctive central fulcra.

5. *Cavinula lapidosa* (Krasske) Lange-Bertalot Fig. 6A

Reference: Lange-Bertalot & Metzeltin (1996) (p. 30, pl. 24, fig. 17: a–b)

Description: Valves rhombic-elliptic with broadly rounded ends, 15–16 x 6–7.5 μm. The axial area is narrow and linear. The central area is “bow-tie” bordered by 5–7 irregularly shortened striae. Striae are curved and radiate throughout, and increase slightly in density toward the apices, (23–) 24–26 in 10 μm. The raphe is filiform, with straight proximal ends and distal ends turned in opposite directions.

Distribution in Egypt: This species is a new record for the Egyptian freshwater diatoms. It was rare in the epilithon of the River Nile-Damietta Branch.

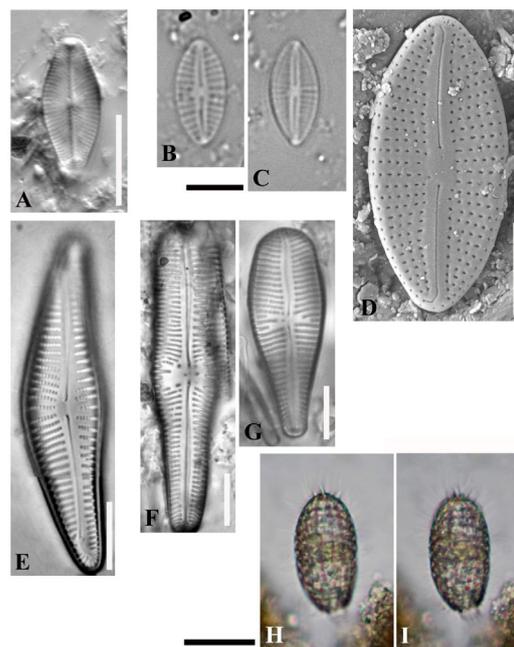


Fig. 6 (A–I). LM and SEM micrographs of some diatoms and the chrysophyte species in the present study; A: *Cavinula lapidosa*, B–D: LM and SEM micrographs of *Craticula subminuscula*, E: *Encyonema neomesianum*, F–G: *Gomphonema laticollum*. H–I: *Mallomonas crassisquama* in different focal planes [Scale bars= 10 μm (Figs A, E–I), 5 μm (Figs B–C), 3 μm (Fig. D)]

General distribution and ecology: Widespread freshwater diatom species but which usually occurs in low abundance (Lange-Bertalot et al., 2017; Tyree, 2018). In the present study, it was found in the nutrient-rich waters of the Damietta Branch of the Nile. Potapova & Charles (2007) reported it as a proxy of low nitrogen conditions. Szczepocka & Rakowska (2015) also highlighted that it usually prefers silica-rich waters.

Comments: Our specimens resemble the valve outline and dimensions, and striae density of the populations from Europe (Lange-Bertalot et al., 2017), and the rivers in the United States (Tyree, 2018). However, future integrative studies on the world populations of this morphospecies might help resolving apparent inconsistencies in the ecological preferences.

6. *Craticula subminuscula* (Manguin) C.E. Wetzel & Ector Fig. 6B–D

Reference: Moser et al. (1998) (p. 49, pl. 154)

Description: Valves elliptic to elliptical-lanceolate with narrowly rounded apices, 8.5–

9.5 x 4.0–5.0µm. Striae are radiate throughout, 26–28 (–30) in 10µm (Fig. 6B–C). In SEM (Fig. 6D), striae are uniseriate, areolae are round or transapically elongated. The axial area is linear, thickened, faintly widened in the center. The raphe is filiform on a slightly elevated sternum. Proximal raphe endings are expanded into small central pores and weakly deflected to the same direction. Distally, raphe endings are short, unilaterally deflected and not passing onto the valve mantle.

Distribution in Egypt: This species is a new record for the Egyptian freshwater diatoms. During this study, it was found in low abundance in the epilithon of the River Nile-Damietta Branch.

General distribution and ecology: Widespread diatom species. It is also well known as eutrathentic species, particularly in electrolyte-rich and heavily polluted waters (Potapova, 2009; Zlatko et al., 2016). During the present study, it was found in the nutrient-rich waters of the Damietta Branch of the Nile.

Comments: This diatom species, and even its basionym *Navicula subminuscula* and homotypic synonym *Eolimna subminuscula*, have not yet been reported in the Egyptian diatom inventory based on the available literature. *Craticula molestiformis* is the morphologically most similar taxon, but the two species can be easily differentiated in terms of valve outline, stria densities and orientation, and the raphe structure (Lange-Bertalot, 2001; Lange-Bertalot et al., 2017).

7. *Encyonema neomesianum* Krammer Fig. 6E

Reference: Krammer (1997) (p. 5, pl. 191, figs 7–9)

Description: Valves dorsiventral, half lanceolate, dorsal margin convex, ventral margin straight and expanded centrally, 47.5–51 x 11–13µm. Valve apices are bluntly rounded. Dorsal and ventral striae are radiate throughout and convergent at the apices, 8–9 at the center, and 10–12 at the apices. Areolae are coarse, easily visible in LM, measuring 21–23 in 10µm. A single stigmoid occurs in line with the central dorsal stria. The axial area is narrow near the apices, and gradually widens towards the mid-valve. Terminal nodules are clearly visible. The raphe slightly lateral, filiform, proximal endings unilaterally deflected towards the dorsal margin, and distal endings evidently curved and deflected towards the ventral margin.

Distribution in Egypt: So far, this diatom species has been documented only two times in Egyptian freshwater habitats. Saleh (2009) recorded its old synonym *Cymbella turgida* var. *pseudogracilis* from the thermal spring “Ain Waleda” in the New Valley Governorate, and Elrefaey (2018) found it as epilithic in the Nile. During the present study, it was found as rarely occurring in the epilithon of the Damietta branch.

General distribution and ecology: Freshwater, alkaliphilous, pollution-tolerant diatom species widely distributed across Africa (Cholnoky, 1958), South America, North America (Patrick & Reimer 1975; Slate & Stevenson, 2007), and Asia (Al-Handal & Al-Shaheen, 2019). During the present study, it was found in the slightly alkaline, nutrient-rich waters of the Damietta Branch of the Nile. The same ecological preferences have been reported by Slate & Stevenson (2007) and Elrefaey (2018). Cholnoky (1958) registered it from weakly acidic freshwater habitats in South Africa.

Comments: Our specimens coincide with the key taxonomic features of the type population (Krammer, 1997). Based on information available on its biogeographical distribution, this diatom species seems to be widely distributed in subtropical and tropical freshwater biotopes across the globe, but has not yet been recorded in Europe (Lange-Bertalot et al., 2017).

8. *Gomphonema laticollum* E. Reichardt Fig. 6F–G

Reference: Reichardt (2001: p. 199, pl. 5, figs 1–14), Levkov et al. (2016: pl. 22, figs 1–15)

Description: Valves clavate, swollen in the median region in particular in larger specimens, and with a little-pronounced constriction between the median region and the apices, 39.5–60 x 12.5–14µm. Apices broadly rounded and bases narrowly rounded. The axial area relatively narrow and linear. The central area delimited by irregularly shortened striae. The raphe sinuous with proximal endings dilated slightly into pores and bent towards the stigma. Striae radiate, uniseriate, composed of areolae clearly visible in LM, 9–11 in 10µm.

Distribution in Egypt: This species was previously recorded in Egypt by Abdel-Hamid et al. (2017) in the El-Salam Canal. In the present study, it was abundant in the epilithon of the River Nile-Damietta Branch.

General distribution and ecology: Widespread freshwater diatom in rivers with a tendency to tolerate nutrient-rich waters heavily affected by organic pollution.

Comments: The diagnostic taxonomic features of our specimens fell within the ranges of the holotype material illustrated by Reichardt (2001). Additionally, the Nile River specimens highly resemble *G. laticollum* morphotype I from the River Zrnovka (Levkov et al., 2016). The relatively more linear shape of *G. laticollum* (Fig. 6F), in particular, distinguishes it from other morphologically similar taxa such as *G. turgidum* and *G. capitatum*. Generally, *G. laticollum* differs from the two latter taxa by the presence of a less pronounced constriction near the valve apices, in addition to valve dimensions and ultrastructure. Further studies on this species complex from different Egyptian biotopes, using SEM approach, should be pursued in the future to refine and delimit the species identification, and also to distinguish it from allied taxa.

9. *Mallomonas crassisquama* (Asmund) Fott Fig. 6H–I

Reference: Kristiansen & Preisig (2007) (p. 65; Fig. 45: a–b)

Description: Cells ovoid-ellipsoid, 13–18 x 8–10 μm. Bristles all over the cell, except posteriorly where caudal scales with conspicuous spines are present.

Distribution in Egypt: This is the first record of this species in Egypt. In the present study, it was found among the other periphytic algae inhabiting the hyper-arid valley “Wadi Al-Naq’at” in the Eastern Desert of Egypt.

General distribution and ecology: Freshwater species widely distributed in temperate and subtropical regions (Kristiansen & Preisig, 2007).

Comments: So far, very little information is available on the diversity and ecological distribution of the genus *Mallomonas* in Egypt, and hence further in-depth studies, using combined SEM and molecular phylogeny, on this little-studied genus and its algal group should be conducted. Shaaban (1994), for instance, only reported *M. caudata* and *M. acaroides* in the River-Nile basin, Lake Nasser and Aswan reservoir. Konsowa (2007) recorded *M. heterospina* and *M. multiunca* from Wadi El-Rayan Lakes.

10. *Hallasia cf. reticulata* (Hallas) Rosenvinge ex Curzda Fig. 7A–K

Reference: Randhawa (1959) (p. 186; fig. 115: a–f)

Description: Vegetative cells 18–20 (–22) x 55–145 μm, with plane end walls and 2 distinctive stellate chloroplasts in each cell (Fig. 7A–E). Each stellate chloroplast with a conspicuous central pyrenoid, and branches radiating in all planes towards the cell wall (Fig. 7F–J). Reproduction not observed.

Distribution in Egypt: This is the first record of this interesting species for Egypt. During the present study, it was found in a good mass growth among other periphytic algae in the hyper-arid valley “Wadi Al-Naq’at” in the Eastern Desert of Egypt.

General distribution and ecology: In the present study, this species was found in typical, alkaline, freshwater conditions (pH: 8.81, ions’ conductivity: 550 μS.cm⁻¹, average values of cations, anions and metals were in the typical range for this pristine Saharan habitat (Table 1). So far, this interesting filamentous streptophyte has a limited biogeographical distribution, having been recorded only in Denmark, Spain, Germany, USA, Pakistan, Britain (Smith, 1933; Randhawa, 1959; Álvarez Cobelas, 1984; Zarina et al., 2007). It therefore not only represents a new record for Egypt but also for the African continent (Fig. 7K).

Comments: Our specimens have longer vegetative cells than the type population (55–145 μm vs. 35–100, respectively). However, other details of the vegetative cells and structure of the stellate chloroplasts highly coincide with the holotype (compare them with Fig. 115:a in Randhawa, 1959; Fig. 355:b in Kadlubowska, 1984). As the structure of peculiar aplanospores and the germination behaviour play a very important role in the delineation of this genus and its species, and these taxonomic features had not been observed in our specimens, we herein proposed it as *Hallasia cf. reticulata*. Worthy of note, we cultivated the materials on the algal growth media Chu #10 and BBM but the filaments did not grow well, and no spores were formed. It is highly recommended to re-investigate this interesting and worldwide rarely-recorded species using a combined polyphasic approach.

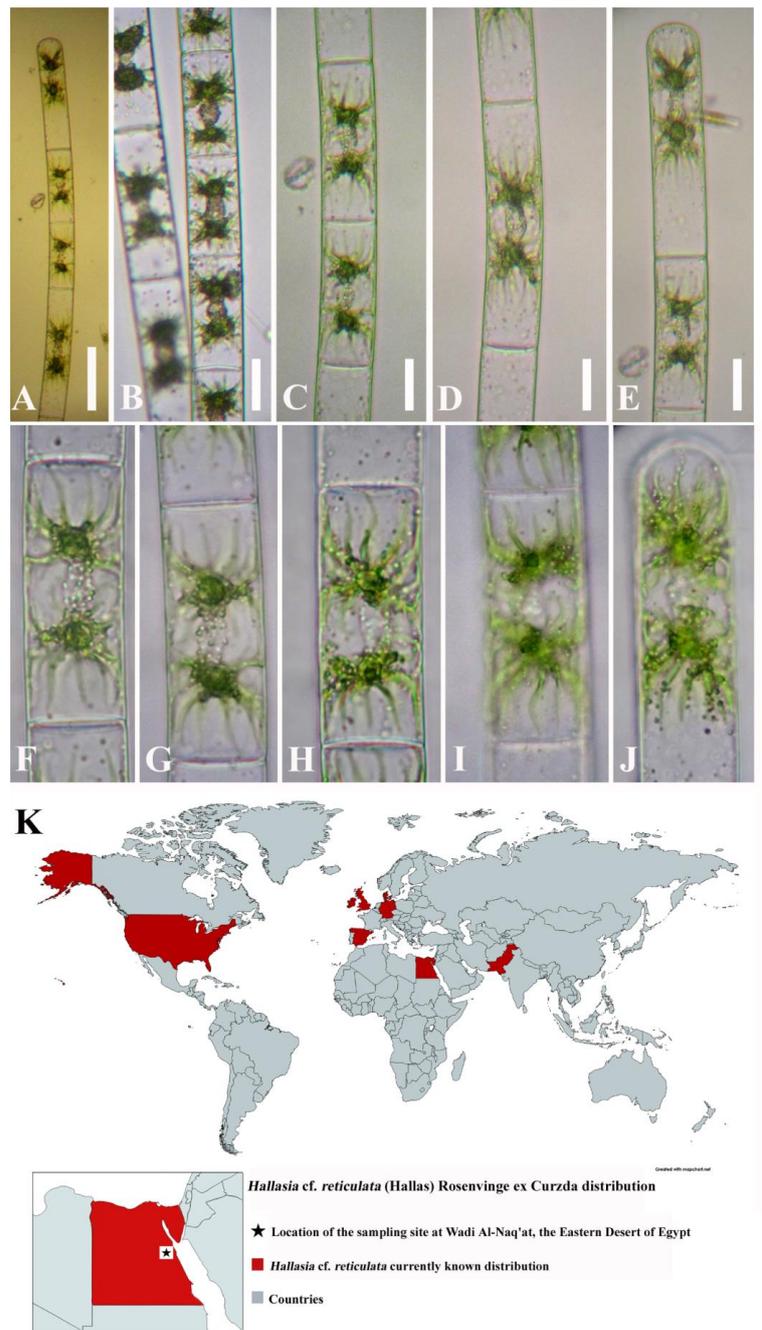


Fig. 7 (A–K). *Hallasia cf. reticulata*; A: Overview of the whole filament, B–E: The vegetative cells, F–J: Close-up view depicting details of the stellate chloroplasts, K: Currently-known worldwide distribution [Scale bars= 50 μ m (Fig. A), 20 μ m (Figs B–J)]

Conclusion

In this study, and based on the available literature, the finding of newly-described and poorly-known cyanobacterial and algal taxa consolidates the hypothesis that our understanding on the Egyptian algal biodiversity is still limited. This is also in obvious agreement with the global assumption that the algal flora of the African continent as a whole

is still largely uncovered and needs more detailed phycological studies (Cantonati et al., 2020b). As advocated for cyanobacteria, and in congruence with previously published data, Komárek (2003) and Hamed (2005) stressed that African cyanobacteria, particularly in the Mediterranean regions, including Egypt, are still poorly known in terms of covering the potential biodiversity. A

recent integrative polyphasic study by Saber et al. (2017a) confirmed the presence of the tropical-to-subtropical heterocytous true-branching cyanobacterium *Westiellopsis prolifica* as a new record for Egypt and improved our knowledge of this species. As regards other algal groups, some species new to science, e.g. *Euastrum elfarafraense* (Desmidiaceae, Streptophyta) (Saber et al., 2018b), and *Seminavis aegyptiaca* (Naviculales, Bacillariophyta) (Saber et al., 2020) have also been recently discovered from different Egyptian biotopes, providing some evidence for the possible occurrence of algal endemism in Egypt, pointing to this country as a shrine of relevant algal and cyanobacterial diversity. With respect to diatoms, our findings confirm the limited knowledge of the diversity of diatom species in the Egyptian habitats. This conclusion derives from the fact that most of the previous Egyptian taxonomic diatom studies depended on the LM only, overlooking many distinctive key characters of the valve ultrastructure, and therefore many diatom taxa were misidentified as cosmopolitan species. Improving our knowledge on the accurate species composition and community structure of diatoms as meaningful proxies would enhance the capability to assess the ecological health status of inland waters due to their high sensitivity and rapid responses (short life cycles) to the hydrology-related variables and human disturbances (van Dam et al., 1994; Potapova & Charles, 2003; Olszyński et al., 2019; Cantonati et al., 2020a and the references therein).

We think that the algal biodiversity of Egypt is of global interest due to the divergence of its habitats, as well as its unique geographical position at the juncture among Europe, Africa, and Asia, thus supporting many interesting and unusual cyanobacterial and algal taxa. Several novel and interesting taxa, including diatoms, are still hidden (Saber, Cantonati & other coauthors, unpublished data), and therefore, more detailed studies, depending upon state-of-the-art classification systems, should be pursued. Accordingly, Vanormelingen et al. (2008) pointed out the necessity of directing much more efforts towards assessing distributional patterns of diatom species using molecular, morphotaxonomic, and ecological data to be able to split and/or confirm the hypothesis of species conspecificity in putatively-cosmopolitan morphospecies.

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Conflict of interests: The authors declare no conflict of interest.

Authors contribution: ME, MC and AAS conceived and designed the structure of the article. ME, AYN, MC and HS performed the literature search and the data analysis. ME, AAS, MC and HS wrote the first draft of the manuscript. ME, AYN, MC and AAS reviewed the manuscript. All authors read and approved the final version of the manuscript.

Ethical approval: Not applicable.

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ملاحظات تصنيفية وبيئية على بعض أنواع الطحالب والطحالب الزرقاء المخضرة الجديدة أو التي نادراً ما تم تسجيلها في أي من مصر أو إفريقيا

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يعد فهم التنوع والتوزيع الجغرافي للطحالب الزرقاء المخضرة والطحالب في مصر تحدياً صعباً للغاية لأنه لا يزال موضوعاً قيد الدراسة لمعالجة هذه الفجوة المعرفية، تم دراسة الصفات المورفولوجية والتصنيفية، وكذلك السمات البيئية، لعشرة أنواع من الطحالب الزرقاء المخضرة والطحالب من بيئات مصرية متنوعة. تم دراسة و تعريف تلك الأنواع باستخدام وسائل تصنيفية حديثة ودقيقة تعتمد على الميكروسكوب الضوئي والميكروسكوب الإلكتروني الماسح. من هذه الأنواع، تعتبر الطحالب الزرقاء المخضرة *Lemmermanniella uliginosa* and *Scytonema myochrous*، دياتومات المياه العذبة *Cyclotella meduanae*, *Cavinula lapidosa* and *Craticula subminuscula*، الطحلب الذهبي وحيد الخلية *Mallomonas crassisquama*، والطحلب الخيطي الأخضر النادر تسجيله على مستوى العالم *Hallasia cf. reticulata* أنواع جديدة لمصر. علاوة على ذلك، يعتبر كل من النوع الأخير وطحلب *L. uliginosa* أيضاً أنواع جديدة للقارة الأفريقية. دياتومات المياه العذبة *Cyclostephanos invisitatus*, *Encyonema neomesianum*, and *Gomphonema laticollum* نادراً ما سجلت في الدراسات المصرية السابقة. بشكل عام معظم الأنواع التي تم تعريفها تستطيع أن تتحمل التركيزات العالية من المغذيات (أنواع دالة على التلوث الغير عضوي المتوسط و القوي)، مما يعكس التأثيرات البشرية المختلفة على البيئات التي تستوطنها. هذه الأنواع المسجلة حديثاً للبيئة المصرية غالباً ما قد تم التغافل عنها في الدراسات المصرية السابقة إما بسبب بيئاتها المعزولة نسبياً، أو لصغر حجمها، أو للوضع التصنيفي والتسميات المعقدة المتبعة. لا تزال هناك أنواع أخرى مثيرة للأهتمام من الطحالب و الطحالب الزرقاء المخضرة لم يتم إكتشافها بعد في مصر، لاسيما في البيئات الصحراوية المعزولة قليلة الدراسة، وبالتالي لاغنى عن مزيد من البحث، باستخدام وسائل وطرق متعددة، لتحقيق تقييم أفضل لتنوع الطحالب الزرقاء المخضرة والطحالب المصرية، ولإنشاء أنظمة تقييم فعالة تعتمد على الطحالب مصممة خصيصاً للبيئات المصرية.