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Adaptive Traits of Bloom Forming *Sphaerospermopsis aphanizomenoides* in a Mediterranean Ramsar Wetland: Morphology, 16S Phylogeny and Nutrient/Light Responses

Aicha ZELLAL[1*], Sid-Ahmed Chawki LAMARA[1], Sidi Mohammed El-Amine ABI-AYAD[1]
Laboratory of Aquaculture & Bioremediation (AquaBior). Department of Biotechnology I.G.M.O Campus, University of Oran 1 Ahmed Ben Bella, Faculty of Natural Sciences and Life, Algeria

[*]**Corresponding Author: zellalaicha@yahoo.fr**

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ABSTRACT

Sphaerospermopsis aphanizomenoides is an invasive cyanobacterial species recently detected in the Macta Marshes, a Ramsar-listed wetland in northwestern Algeria recognized for its ecological importance to Mediterranean biodiversity. In recent years, this area experienced advanced eutrophication affecting numerous watercourses and small lakes. The present study provides a morphological, molecular and physiological characterization of *S. aphanizomenoides* strain isolated from a summer bloom in the marshes, where chlorophyll a concentration reached 141.6µg/l, indicating an eutrophic aquatic ecosystems with high phytoplankton biomass. Morphological identification supported by 16SrDNA gene sequencing confirmed the species identity. Physiological assays revealed that the strain can grow in nitrate-free media, whereas phosphate limitation significantly reduced its development. Moreover, the ability of the strain to grow under complete darkness highlights its adaptive strategies to low light conditions. These findings demonstrate the remarkable adaptability of *Sphaerospermopsis aphanizomenoides* and its potential role in algal blooms, underscoring the need for further research into its ecological impact and proliferation mechanisms.

INTRODUCTION

Global climate change and eutrophication are increasingly influencing the distribution and seasonal dynamics of cyanobacterial communities worldwide **(Kleinteich *et al.*, 2024)**. Certain harmful filamentous cyanobacteria that were initially identified in the tropics are also migrating to temperate regions like North America, Europe, and Northeast Asia **(Kim *et al.*, 2020)**, although the spread of cyanobacteria to new areas is mainly facilitated by human activities, while natural means such as birds, rivers, or wind also play a crucial role **(Curren & Leong, 2020)**. Aphanizomenonaceae is a newly proposed family of filamentous cyanobacteria that includes 12 planktic genera; the morphological characteristics of this family include the formation and distribution of

heterocysts and akinetes along the trichomes (**Célia-Sant'Anna *et al.*, 2019)**. However, the application of contemporary molecular tools and phylogenetic interference accelerated the revision and expansion of cyanobacterial taxonomy in the early twenty-first century **(Kaštovský, 2023)**. Therefore, recent investigations, based on the 16SrDNA gene sequence and the production of secondary metabolites **(Zapomělová *et al.*, 2009)**, reclassified the traditional nostocacean genus *Anabaena* to the newly established genus *Sphaerospermopsis* **(Zapomělová *et al.*, 2011)**. The species is recognized by trichomes with large spherical akinetes and short cylindrical to spherical heterocysts situated adjacent to or between the akinetes **(Hindak *et al.*, 2000)**.

These Nostocales species have the highest affinity for phosphorus, which gives them a significant competitive advantage during their invasion **(Sukenik *et al.*, 2012)**. These invasive species can produce cyanotoxins that can be hazardous to the liver, brain, skin, and kidneys **(Napiórkowska *et al.*, 2023)**. Due to their wide distribution, bioaccumulation capacity and toxic effects, cyanotoxins like cylindrospermopsin and microcystins are the most relevant worldwide **(Weralupitiya *et al.*, 2022)**. Recent studies by **Hinojosa *et al.* (2023)** and **Plata-Calzado (2023)** have shown that these toxins exhibit dual activity, affecting both cells and the nervous system. Furthermore, the thermostable tricyclic structure of some cyanobacterial toxins presents a significant challenge for water treatment plants, as conventional methods are often unable to remove toxins effectively **(Wu *et al.*, 2015)**. In Europe, *Sphaerospermopsis aphanizomenoides* formerly known as *Aphanizomenon sphaericum* Kisselev is regarded as an invasive species of cyanobacterium which gained attention due to its potential to produce harmful cyanotoxins **(Sabour *et al.*, 2005; Kaštovský *et al.*, 2010; Ballot *et al.*, 2014**) and its ability to thrive in different environments **(Zapomělová *et al.*, 2009, 2012)**. The growth dynamics of this species are affected by environmental factors, particularly nutrient availability and light intensity, which play key roles in its development and proliferation **(Wiedner *et al.*, 2007)**.

Given the increasing frequency of cyanobacterial blooms and the global spread of invasive species, several reports have documented the presence of cyanobacteria in socio-economically important ecosystems in Algeria, particularly in the eastern part of the country **(Saoudi *et al.*, 2017; Touati *et al.*, 2019; Benredjem *et al.*, 2022, 2023)**. In the northwestern region, the waters of the Macta Marshes (RAMSAR site) play a critical role in irrigation, livestock, nutrient retention and support for aquatic fauna and flora, as well as in recreational activities. Despite the marshes significance, research in this area has largely focused on hydrological analysis and the impact of climate change on the Macta wetland **(Katelyn *et al.*, 2024)**. To our knowledge, no studies have been conducted to assess the presence of this invasive cyanobacterium and research on *Sphaerospermopsis aphanizomenoides* in this region remains limited. The main objectives of this study were to confirm the occurrence of *S. aphanizomenoides* in the Macta Marshes, and perform its

morphological, molecular and physiological characterization in order to contribute to the management of eutrophication-affected aquatic ecosystems.

MATERIALS AND M*ET* HODS

1. Site characterization

The water quality of the Macta Marshes has declined due to increasing agricultural runoff, industrial effluents and untreated domestic discharges. As a result, these anthropogenic pressures have caused nutrient enrichment and eutrophication. Recent studies have also identified overexploitation and hydrological closure of the Macta wetland as major contributors to environmental degradation **(Kherbache & Molle, 2023)**. Samples were collected from the Macta Marshes (35˚47'04.81"N, 0˚06'29.73"O, Fig. 1) during summer (August, 2021). Water samples were collected from a depth of 4–20cm of the water surface in sterile glass bottles. Plankton samples were taken using a plankton net (20µm-mesh size). A pooled-sample aliquot (250mL) was preserved immediately with Lugol's solution for cyanobacterial identification. The trophic state of the marshes and phytoplankton biomass was quantified as chlorophyll a concentration (µg L^{-1}). **(Meriluoto *et al.*, 2005)**. Salinity, pH, and temperature were measured *in situ* with a multiparameter probe (ExStik Series EC500); samples were then stored and transported in the dark at 4°C. These data contributed to a better understanding of the strain's natural habitat and guided the optimization of culture conditions. Laboratory analyses were conducted in the Aquaculture and Bioremediation Laboratory, Biotechnology Department, University of Oran 1 (Algeria).

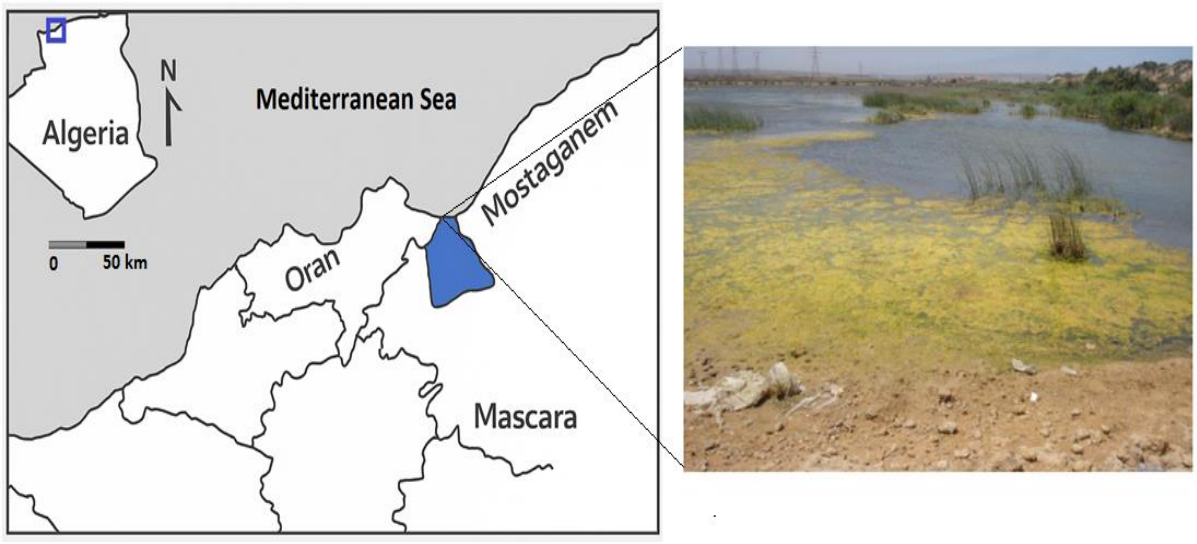

Fig. 1. Site of cyanobacterial bloom sampling in Macta Marshes, northwestern Algeria, during August 2021

2. Morphological characterization

Cyanobacterial isolates were purified by serial dilution and cultured in BG-11 medium optimized for growth under axenic laboratory conditions. The strains were maintained in designated liquid media at 28 ± 2°C under ≈2500 Lux white fluorescent light with a 12:12h light-dark cycle for 15 days **(Lee *et al.*, 2014)**. Morphological features were examined using an Olympus light microscope at magnifications of 100×, 400×, and 1000×, focusing on colony shape, cell diameter, and mucilage characteristics **(Zapomělová *et al.*, 2009; McGregor *et al.*, 2018)**.

3. Molecular characterization

Cyanobacterial cultures were harvested at exponential growth phase by centrifugation of 5mL aliquots at 15,000 × g for 1 minute. DNA was extracted from the cell pellets using the Plant Genomic DNA Extraction Kit and stored at −20°C. DNA quality and integrity were verified by electrophoresis on a 1.0% agarose gel. The 16S rRNA gene was amplified via PCR using primers 27F1 (AGAGTTTGATCCTGGCTCAG) and 809R (GCTTCGGCACGGCTCGGGTCGATA) targeting a 780 bp fragment, following **Jungblut *et al.* (2006)**. PCR reactions (25µL total volume) included 50ng template DNA, 0.3µM primers, 1× Thermopol buffer, 200 µM dNTPs, and 0.025U/ µL Taq polymerase, thermocycled on an Applied Biosystems ABI 9700. Amplicons were visualized by ethidium bromide staining after electrophoresis on 0.8% agarose gels under UV light. Bidirectional Sanger sequencing was performed by GENWIZ (Leipzig, Germany). Resulting sequences were compared to NCBI references and deposited in GenBank (accession no. PP999716). Phylogenetic analyses were conducted with MEGA v7.0.21 using the neighbor-joining method **(Saitou & Nei, 1987)**.

4. Physiological characterization

Cyanobacterial isolates were cultivated in BG-11 medium with modified nutrient conditions to assess the effects of nutrient absence and light limitation. Three experimental regimes were used: BG-11 without nitrate (NO_3^-), BG-11 without phosphate (PO_4^{3-}), and complete darkness; a control consisted of standard BG-11. Cultures were maintained in defined liquid media at 25 ± 1°C under approximately 2,500 lux of white fluorescent light with a 12h light/ 12h dark cycle for 10 days. Chlorophyll-a was measured every two days to monitor growth, using the Burnison extraction with dim*et* hyl sulfoxide (DMSO) and a 1.0% agarose gel check for integrity. Chlorophyll-a was quantified following **Seely *et al.* (1972)**. Each condition was run in triplicate. Chlorophyll-a (mg L−1) was calculated as A × D × F, where A is absorbance at 666 nm, F is the conversion factor (11.3), and D is the extract volume/sample volume. Statistical analysis of daily chlorophyll-a changes was performed with Statistica 12, with a post hoc Tukey test ($P< 0.05$) to identify significant differences. The physiological assays were conducted to identify the environmental factors limiting toxic cyanobacteria growth, providing critical insights for mitigating eutrophication in aquatic ecosystems.

RESULTS

1. Site parameters assessment

The potential environmental factors and their observed ranges in the study area were measured to provide a snapshot of the physical and chemical conditions influencing the aquatic ecosystem at the time of sampling. In August (2021), the chlorophyll-a concentration in the water was measured at 141.6 µg L^{-1}, indicating a high phytoplankton biomass. Simultaneously, the pH was 8.2, reflecting a slightly alkaline environment, while the temperature reached 28.4°C. Additionally, the salinity measured 6.2 ppt suggested moderately brackish conditions.

2. Morphological characterization

Table (1) presents morphological characteristics of the isolate, with corresponding photomicrographs shown in Fig. (2), following the morphological features of **Zapomelovà *et al.* (2009)**.

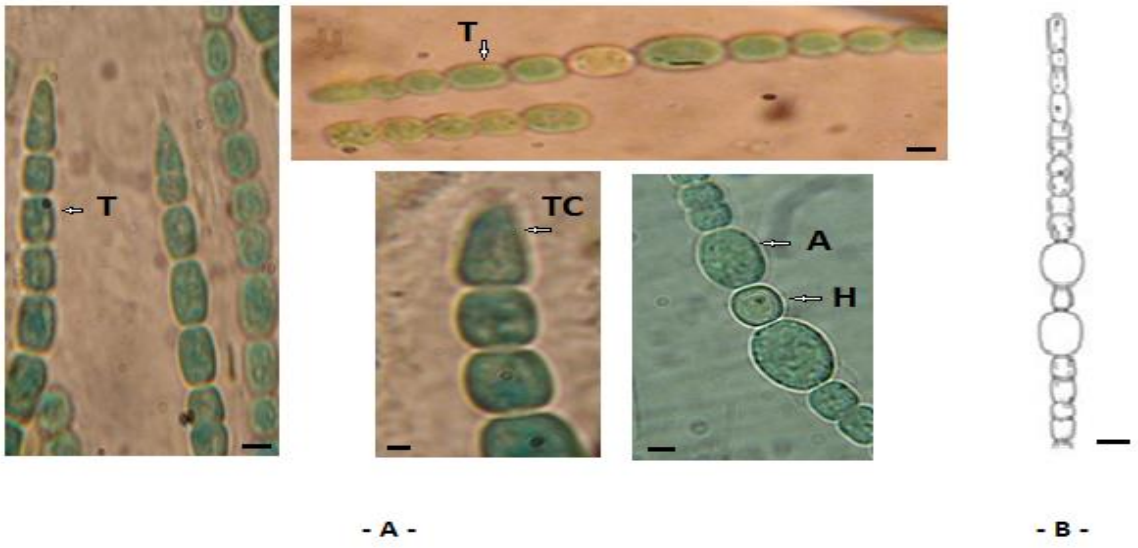

Fig. 2. Morphology of *Sphaerospermopsis aphanizomenoides* (CYA2) showing: (A) Light micrographs showing different morphological forms of the isolate from Macta Marshes: trichomes (T), akinetest (A), heterocysts (H), and terminal cells (TC). (B) Illustrations of *S. aphanizomenoides* (Forti) **(Zapomělová *et al.* (2009)**. Scale bar = 10 µm.

Table 1. Morphological characteristics of *S. aphanizomenoides* isolate from the Macta Marshes. W- width (µm)

Isolate	Trichome type	Vegetative cells	Heterocytes	Akinetest	Terminal cells
Sphaerospermopsis aphanizomenoides **Cya2 (PP999716)**	straight	barrel shaped to cylindrical W=4.8 – 7.5 µm	spherical or slightly elongated W=6.3 − 8.7 µm	spherical or widely oval W= 9.5− 14 µm	slightly elongated rounded ends or conical

3. Molecular characterization

- **DNA quality assessment**

Genomic DNA was successfully extracted. The integrity and quality of DNA were checked on 1.0 % agarose gel (Fig. 3).

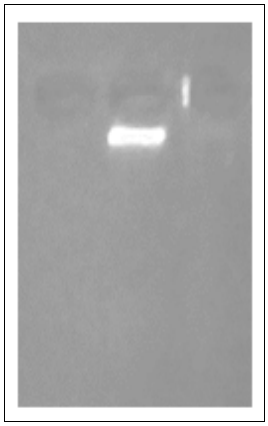

Fig. 3. Gel electrophoresis of genomic DNA extraction from isolate of *Sphaerospermopsis aphanizomenoides*, 1% agarose gel

- **16SrDNA gene analysis**

In this study, cyanobacteria were identified using polymerase chain reaction (PCR) targeting the 16SrDNA gene. Gel electrophoresis analysis revealed distinct amplicon patterns from sample, with DNA extracts showing sizes of 780bp for 16SrDNA (Fig. 4).

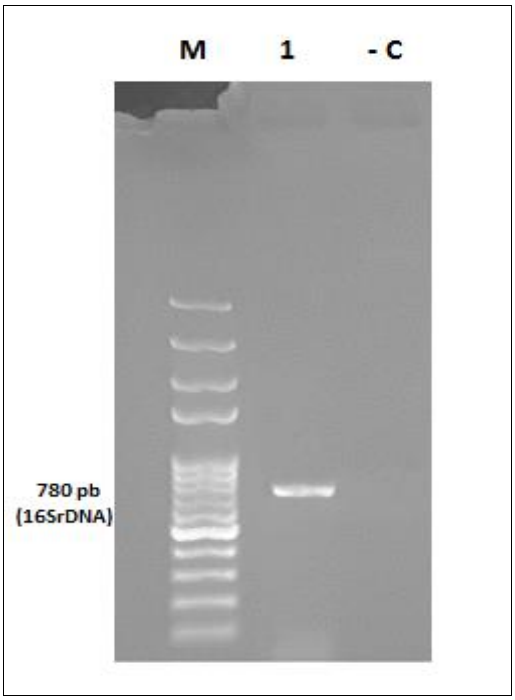

Fig. 4. PCR product the band size 780 bp of 16SrDNA. **M**: Marker (DNA ladder 100 pb), **- C:** Negative control, **1**: *Sphaerospermopsis aphanizomenoides*

- **Phylogenetic study**

BLAST analysis showed high sequence similarities ranging from 97.65 to 97.99% between the 16S rDNA gene sequence of cyanobacterial isolate from the Macta Marshes and strain sequence from the Nostocales order accessible in GenBank (Table 2).

Table 2. 16S rDNA gene-sequence-based identity (%) between the cyanobacterial isolates from the Macta Marshes and their closest match available in Genbank (NCBI)

Isolates	Closest Match (Accession Number)	Query Coverage (%)	Percent Identity (%)
***Sphaerospermopsis aphanizomenoides* Cya2 (PP999716)**	*Sphaerospermopsis aphanizomenoides* IFCC-AA05 (KY077261.1)	100	97.65
	Sphaerospermopsis reniformis NIES-1943 (LC455633.1)	98	97.99
	Sphaerospermopsis aphanizomenoides NRERC-603 (MT294023.1)	98	97.86
	Sphaerospermopsis aphanizomenoides NRERC-607 (MT294021.1)	98	97.74

The phylogenetic tree analysis of 16SrDNA sequences (Fig. 5) revealed that the isolate *Sphaerospermopsis aphanizomenoides* CYA2 grouped within a well-supported

cluster containing reference strains of the same species, including *S. aphanizomenoides* IFCC-AA05 (Turkey), *S. reniformis* NIES-1943 (Japan), and *S. aphanizomenoides* NRERC-603 and NRERC-607 (South Korea), all retrieved from the GenBank database.

Fig. 5. Phylogenetic analysis of *Sphaerospermopsis aphanizomenoides* (CYA2). Neighbor-joining tree based on 16S rDNA sequences showing the relationship of the Macta Marshes isolate (*S. aphanizomenoides* CYA2) to reference cyanobacterial strains from GenBank. The uncultured cyanobacterium JF90897 was used as the outgroup.

4. Physiological characterization

Table (3) presents the results of chlorophyll-a production under the three tested conditions. The results show that the absence of nitrate induced a significant increase in chlorophyll-a production, peaking at Day 6 (15.4 ± 0.02 mg/L), before gradually decreasing. The absence of phosphate led to stagnation in chlorophyll-a production, with values close to the initial control, while cultures in total darkness exhibited no significant increase in biomass, remaining relatively stable throughout the experiment.

Table 3. Chlorophyll-a concentration (mg/L) under different conditions

Conditions	J0	J2	J4	J6	J8	J10
Control	5.15 ± 0.05 ^{a}	8.54 ± 0.34 ^{b}	12.05 ± 0.55 ^{c}	19.54 ± 0.02^{d}	9.44 ± 0.02 ^{b}	7.29 ± 0.10 ^{a}
Nitrate-Free	5.32 ± 0.05 ^{a}	7.27 ± 0.46 ^{b}	10.5 ± 0.02 ^{c}	15.4 ± 0.02^{d}	7.3 ± 0.02 ^{b}	4.6 ± 0.05 ^{a}
Phosphate-Free	5.24 ± 0.07 ^{a}	3.68 ± 0.17 ^{a}	4.00 ± 0.19 ^{a}	6.44 ± 0.10 ^{b}	5.71 ± 0.11 ^{b}	4.77 ± 0.12 ^{ab}
Total darkness	5.10 ± 0.02 ^{a}	4.02 ± 1.22 ^{b}	4.00 ± 0.19 ^{b}	3.97 ± 0.24 ^{b}	4.78 ± 0.01 ^{ba}	5.22 ± 0.05 ^{a}

Values are presented as mean±standard deviation. Letters (a, b, c) indicate significant differences (P< 0.05) within each condition.

DISCUSSION

1. Site characterization

The summer temperature recorded at the Macta Marshes was 27.9°C ± 0.6 (August 28.4°C), showing that *S. aphanizomenoides* is a more effective competitor than native species at higher temperatures **(McGregor *et al.,* 2018)**. Laboratory experiments indicate that this species thrives at 20- 30°C, although optimal temperatures vary between strains **(Savadova-Ratkus *et al.,* 2021)**. The chlorophyll a concentration measured at the site is 141.6µg/ L, which indicates that the area is eutrophic and confirms previous hydrological studies **(Lakhdari, 2021)**. Moreover, cyanobacteria are often associated with eutrophic environments where water transparency is low, including *S. aphanizomenoides* **(Budzyńska *et al.,* 2017)**. However, a significant correlation ($P< 0.05$) was observed between the abundance of *Sphaerospermopsis aphanizomenoides* and chlorophyll-a concentration **(Figueiredo *et al.,* 2022)**, consistent with observations in the Macta Marshes, indicating that eutrophic conditions strongly favor the proliferation of this species. The *in situ* pH of 8.2 lies within the optimal growth range reported for *S. aphanizomenoides* (7.0–9.4) **(Budzyńska *et al.,* 2019)**, suggesting that local alkalinity levels are conductive to its growth. Likewise, the salinity, measurement at 6.2 ppt, reflects a slightly brackish environment, which may further support the species ecological success. Consistent with these field conditions, laboratory experiments have shown that *S. aphanizomenoides* can withstand NaCl concentrations between 100 and 800 mmol L^{-1} **(Usmonkulova *et al.,* 2022)**; nevertheless, fluctuations in salinity are known to significantly influence its growth **(Šuikaitė *et al.,* 2023)**, highlighting the combined influence of eutrophic conditions and moderate salinity on the species proliferation.

2. Morphological characterization

Ecosystems functioning relies on dynamic interactions among species driven by nutrient cycling while environmental disturbances can alter these relationships, favoring opportunistic taxa and initiating successional processes **(Muthukumar *et al.,* 2007)**. However, *Sphaerospermopsis aphanizomenoides* is increasingly reported as an emrging cyanobactrium associated with harmful blooms **(Budzyńska *et al.,* 2017)**. In the present study, a polyphasic approach combining optical microscopy, culture-based characterization and 16SrDNA gene sequencing was used to identify and describe cyanobacterial isolates from the Macta Marshes, with the isolated strain of *S. aphanizomenoide* exhibiting morphological traits consistent with those previously reported **(Zapomělová *et al.,* 2008; Stüken *et al.,* 2009; Komárek *et al.,* 2013; Ballot *et al.,* 2014)**. However, compared with descriptions by **Zapomělová *et al.* (2008)** and **Miller *et al.* (2022)**, our isolate displayed wider vegetative cells, heterocysts and akinetes (4.8–7.5 µm, 6.3–8.7 µm, and 9.5–14 µm, respectively). This morphological

variability likely reflects adaptive responses to environmental and culture conditions, including nutrient availability, temperature and light intensity.

3. Molecular characterization

For the current study, the 16S rDNA gene raw forward and reverse sequences were assembled and subsequently submitted to the GenBank database under accession number PP999716. However sequence of the cyanobacteria isolate from the Macta Marshes and the sequences of Nostocales strains that are available in GenBank showed strong sequence similarities, ranging from 97.65 to 97.99%, with a query coverage of 100%, according to BLAST analysis. The closest match was obtained with *S. aphanizomenoides* IFCC-AA05 (GenBank accession no. KY077261.1). These results confirm that the morphological and phylogenetic identifications are consistent, indicating that the studied isolate belongs to *S. aphanizomenoides*, with only minor intraspecific genetic variation observed. The correspondence with *S. reniformis* is also interesting, suggesting a close phylogenetic relationship or conserved sequences. Additionally, a typical invasive Nostocalean cyanobacterium, *S. aphanizomenoides* has been reported in various parts of the world, including South America **(Bittencourt *et al.*, 2011)**, Africa **(Cirés & Ballot, 2016)** and Asia **(Wu *et al.*, 2016)**. Researchers have reported finding *S. reniformis* and *S. aphanizomenoides* in various countries and these two species are thought to be capable of producing toxins **(Kaštovský *et al.*, 2018)**, responsible for inhibiting the synthesis of proteins and glutathione, which can have hepatotoxic, cytotoxic, neurotoxic, and dermatotoxic effects **(Rodríguez *et al.*, 2023)**. According to the study of **Cordeiro *et al.* (2022)**, two Nostocalean strains, BACA0025 and BACA0031, tested positive for production of toxins and was confirmed by ESI-LC-MS/MS/MS. In recent decades, many parts of the world have experienced warmer springs, which has increased the chances of earlier cyanobacterial blooms **(Qin *et al.*, 2021)**. Nonetheless high temperatures and nutrient loading favor cyanobacterial harmful algal blooms (CHABs) **(Van de Waal *et al.*, 2023)**.

4. Physiological characterization

Sphaerospermopsis aphanizomenoides appears to have spread from subtropical/tropical regions to temperate areas, where it is currently classified as an "alien" or "invasive" species. Despite this fact, Nostocalean cyanobacteria can effectively survive by forming akinetes, which can germinate and grow in the unfavorable conditions like winter season **(Ho *et al.*, 2024)**. Although our results indicate significant growth even without nitrate, research has highlighted that species such as *Aphanizomenon gracile* and *Sphaerospermopsis aphanizomenoides* have exhibited high growth rates under varying nutrient conditions, underscoring their adaptability **(Savadova *et al.*, 2021)**. This could explain why our cyanobacterial species continued to grow efficiently under "Nitrate-Free" conditions. In addition, the absence of phosphate strongly limits the growth of cyanobacteria, which is supported by several recent studies. For instance, a study

revealed that phosphate is often the limiting nutrient in freshwater ecosystems, and its addition can significantly increase cyanobacterial biomass **(Kramer *et al.*, 2022)**. This is corroborated by another research that demonstrated that cyanobacteria, such as *Dolichospermum*, require not only phosphate but also an adequate supply of nitrogen to maximize their growth. However, the present investigation indicate that light is essential for the growth of cyanobacteria, which is supported by a study that observed fluctuations in temperature and light conditions influence the growth dynamics and toxin production in cyanobacteria **(Savadova *et al.*, 2021)**. The ability of cyanobacteria to adapt to conditions of low nutrient availability has been documented in several studies. For example, some species exhibit a preference for storing phosphorus rather than using it immediately for growth, allowing them to survive in environments where nutrients are limited **(Burford *et al.*, 2023)**. This could explain why our results show variability in the response of cyanobacteria to different nutrient conditions.

CONCLUSION

This study provides a comprehensive characterization of *Sphaerospermopsis aphanizomenoides* isolated from a bloom in the Macta Marshes, a wetland in northwestern Algeria. Morphological identification supported by 16SrDNA gene sequencing confirmed the taxonomic position of the isolate. Physiological assays demonstrated that the strain can grow in nitrate-free medium, while phosphate limitation significantly restricted its development. These species also exhibited the ability to grow under complete darkness, albeit at a reduced rate. These findings highlight the ecological plasticity and resilience of *S. aphanizomenoides,* which may explain its successful establishment in eutrophic aquatic environments. Understanding these adaptive traits is crucial for predicting and managing future cyanobacterial blooms under changing environmental conditions. Furthermore, the occurrence of this invasive species in the region underscores its ongoing expansion and ecological significance in North African wetlands.

REFERENCES

Ballot, A.; Sandvik, M.; Rundberget, T.; Botha, C.J. and Miles, C.O. (2014). Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. *Marine and Freshwater Research*, *65*(2), 175–189. https://doi.org/10.1071/mf13153

Benredjem, L.; Berredjem, H.; Abdi, A.; Casero, M.C.; Quesada, A.; Fosso, B.; Marzano, M.; Pesole, G.; Azevedo, J. and Vasconcelos, V. (2022).

Morphological, molecular and biochemical study of cyanobacteria from a eutrophic Algerian reservoir (Cheffia). *SPR*, *29*, 27624–27635.

Benredjem, L.; Morais, J.; Hentschke, G.S.; Abdi, A.; Berredjem, H. and Vasconcelos, V. (2023). The first polyphasic study of cyanobacterial isolates from the Cheffia reservoir (Algeria) reveals a genotype of toxic picocyanobacteria. *Microorganisms*, *11*(11), 2664. https://doi.org/10.3390/microorganisms11112664

Bittencourt-Oliveira, M.d.C.; Piccin-Santos, V.; Kujbida, P. and Moura, A.d.N. (2011). Cylindrospermopsin in Water Supply Reservoirs in Brazil Determined by Immunochemical and Molecular Methods. *Journal of Water Resource and Protection*, *3*(6), 349–355. https://doi.org/10.4236/jwarp.2011.36044

Budzyńska, A.; Chmura, D. and Gołdyn, R. (2017). Domination of invasive Nostocales (Cyanoprokaryota) at 52°N latitude. *Phycological Research*, *65*(4), 322–332. https://doi.org/10.1111/pre.12188

Budzyńska, A.; Rosińska, J.; Pełechata, A.; Toporowska, M.; Napiórkowska-Krzebietke, A.; Kozak, A.; Messyasz, B.; Pęczuła, W.; Kokociński, M. and Pawlik-Skowrońska, B. (2019). Environmental factors driving the occurrence of the invasive cyanobacterium *Sphaerospermopsis aphanizomenoides* (Nostocales) in temperate lakes. *Science of The Total Environment*, *650*, 1338–1347. https://doi.org/10.1016/j.scitotenv.2018.09.144

Burford, M.A.; Willis, A.; Xiao, M.; Prentice, M.J. and Hamilton, D.P. (2023). Understanding the relationship between nutrient availability and freshwater cyanobacterial growth and abundance. *Inland Waters*, *13*(2), 143-152. https://doi.org/10.1080/20442041.2023.2204050

Célia-Sant'Anna, L.; Gama, W.A.; Rigonato, J.; Corrêa, G.; Mesquita, M.C.B. and Marinho, M.M. (2019). Phylogenetic connection among close genera of Aphanizomenonaceae (Cyanobacteria): *Amphiheterocytum* gen. nov., *Cylindrospermopsis* and *Sphaerospermopsis*. *Algal Research*, *37*, 205–214. https://doi.org/10.1016/j.algal.2018.11.026

Cirés, S. and Ballot, A. (2016). A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the Nostocales (cyanobacteria). *Harmful Algae*, *54*, 21–43. https://doi.org/10.1016/j.hal.2015.09.007

Cordeiro, R.; Luz, R.; Azevedo, J.; Vasconcelos, V.; Gonçalves, V. and Fonseca, A. (2022). Expansion of Cylindrospermopsin in the Azores: Evidence for New Producing Taxa. *The 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins*. https://doi.org/10.3390/blsf2022014014

Curren, E. and Leong, S.C.Y. (2020). Natural and anthropogenic dispersal of cyanobacteria: a review. *Hydrobiologia*, *847*(13), 2801–2822. https://doi.org/10.1007/s10750-020-04286-y

Figueiredo, D.; Lopes, A.R.; Pereira, M.J.; Polónia, A.R.M.; Castro, B.B.; Gonçalves, F.; Gomes, N.C.M. and Cleary, D.F.R. (2022). Bacterioplankton Community Shifts during a Spring Bloom of *Aphanizomenon gracile* and *Sphaerospermopsis aphanizomenoides* at a Temperate Shallow Lake. *Hydrobiology*, *1*(4), 499–517. https://doi.org/10.3390/hydrobiology1040030

Hindak, F. (2000). Morphological variation of four planktic nostocalean cyanophytes—members of the genus *Aphanizomenon* or *Anabaena*. *Hydrobiologia*, *438*, 107–116. https://doi.org/10.1023/a:1004118213936

Hinojosa, M.G.; Cascajosa-Lira, A.; Prieto, A.I.; Gutiérrez-Praena, D.; Vasconcelos, V.; Jos, A. and Cameán, A.M. (2023). Cytotoxic Effects and Oxidative Stress Produced by a Cyanobacterial Cylindrospermopsin Producer Extract versus a Cylindrospermopsin Non-Producing Extract on the Neuroblastoma SH-SY5Y Cell Line. *Toxins*, *15*(5), 320. https://doi.org/10.3390/toxins15050320

Hinojosa, M.G.; Gutiérrez-Praena, D.; López, S.; Prieto, A.I.; Moreno, F.J.; Jos, A. and Cameán, A.M. (2023). Toxic effects of the cylindrospermopsin and chlorpyrifos combination on the differentiated SH-SY5Y human neuroblastoma cell line. *Toxicon*, *227*, 107091. https://doi.org/10.1016/j.toxicon.2023.107091

Ho, H.I.; Park, C.H.; Yoo, K.E.; Kim, N.Y. and Hwang, S.J. (2024). Survival and development strategies of cyanobacteria through akinete formation and germination in the life cycle. *Water*, *16*(5), 770. https://doi.org/10.3390/w16050770

Jungblut, A.-D. and Neilan, B.A. (2006). Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. *Archives of Microbiology*, *185*(2), 107–114. https://doi.org/10.1007/s00203-005-0073-5

Kaštovský, J. (2023). Welcome to the jungle!: An overview of modern taxonomy of cyanobacteria. *Hydrobiologia*. https://doi.org/10.1007/s10750-023-05356-7

Kaštovský, J.; Hauer, T.; Mareš, J.; Krautová, M.; Bešta, T.; Komárek, J.; Desortová, B.; Hetesa, J.; Hindáková, A.; Houk, V. and Zapomělová, E. (2010). A review of the alien and expansive species of freshwater cyanobacteria and algae in the Czech Republic. *Biological Invasions*, *12*(10), 3599–3625. https://doi.org/10.1007/s10530-010-9754-3

Katelyn, B.B.; Ryan, S.W.; Christopher, S.W.; Sitoki, L.; James, N.; Omondi, R.; Achiya, J.; Getabu, R.M.; McKay, G. and Bullerjahn, N.S.F.-I.R.E.S. (2024). Bacterial community and cyanotoxin gene distribution of the Winam Gulf, Lake Victoria, Kenya. *Environmental Microbiology Reports*. https://doi.org/10.1111/1758-2229.13297

Kherbache, N. and Molle, F. (2023). Causes and consequences of the Macta basin closure, Algeria. *International Journal of Water Resources Development*, *39*(3), 382-403.

Kleinteich, J.; Frassl, M.A.; Schulz, M. and Fischer, H. (2024). Climate change triggered planktonic cyanobacterial blooms in a regulated temperate river. *Scientific Reports*, *14*(1), 16298.

Kim, Y.-J.; Park, H.-K. and Kim, I.-S. (2020). Invasion and toxin production by exotic nostocalean cyanobacteria (*Cuspidothrix*, *Cylindrospermopsis*, and *Sphaerospermopsis*) in the Nakdong River, Korea. *Harmful Algae*, *100*, 101954. https://doi.org/10.1016/j.hal.2020.101954

Komárek, J. (2013). *Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota*. 3rd part: Heterocytous genera. Springer Spektrum. https://doi.org/10.1007/978-3-8274-2737-3

Kramer, B.J.; Jankowiak, J.G.; Nanjappa, D.; Harke, M.J. and Gobler, C.J. (2022). Nitrogen and phosphorus significantly alter growth, nitrogen fixation, anatoxin-a content, and the transcriptome of the bloom-forming cyanobacterium *Dolichospermum*. *Frontiers in Microbiology*, *13*, 955032. https://doi.org/10.3389/fmicb.2022.955032

Kumar, S.; Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870-1874.

Lakhdari, F.E.T.Z.; Benhanifia, S.K. and Mouassa, S. (2021). Evaluation of water quality and risk of water level declines in semi-arid wetland: case of study in the Macta marshes, Algeria.

Lee, E.; Ryan, U.; Monis, P.; McGregor, G.B.; Bath, A.; Gordon, C. and Paparini, A. (2014). Polyphasic identification of cyanobacterial isolates from Australia. *Water Research*, *59*, 248–261. https://doi.org/10.1016/j.watres.2014.04.023

McGregor, G.B. (2018). Freshwater Cyanobacteria of North-Eastern Australia: 3. Nostocales. *Phytotaxa*, *359*(1), 1–166. https://doi.org/10.11646/phytotaxa.359.1.1

Meriluoto, J.; Codd, G.A.; Reilly, M.; Metcalf, J.S.; Spoof, L.; Sjövall, O.; Hall, T.; Izydorczyk, K.; Lindholm, T. and Tarczynska, M. (2005). Toxic: cyanobacterial monitoring and cyanotoxin analysis.

Miller, S.R.; Abresch, H.E.; Baroch, J.J.; Jacob, C.K.F.; Garber, A.I.; Oman, A.R. and Ulrich, N.J. (2022). Genomic and Functional Variation of the Chlorophyll d-Producing Cyanobacterium *Acaryochloris marina*. *Microorganisms*, *10*(3), 569. https://doi.org/10.3390/microorganisms10030569

Muthukumar, C.; Muralitharan, G.; Vijayakumar, R.; Panneerselvam, A. and Thajuddin, N. (2007). Cyanobacterial biodiversity from different freshwater ponds of Thanjavur, Tamilnadu (India). *Acta Botánica Malacitana*, *32*, 1-10.

Napiórkowska-Krzebietke, A.; Dunalska, J. and Bogacka-Kapusta, E. (2023). Ecological Implications in a Human-Impacted Lake—A Case Study of Cyanobacterial Blooms in a Recreationally Used Water Body. *International*

Journal of Environmental Research and Public Health, *20*(6), 5063. https://doi.org/10.3390/ijerph20065063

Plata-Calzado, C.; Díez-Quijada, L.; Medrano-Padial, C.; Prieto, A.I.; Cameán, A.M. and Jos, A. (2023). In Vitro Mutagenic and Genotoxic Assessment of Anatoxin-a Alone and in Combination with Cylindrospermopsin. *Toxins*, *15*(7), 458. https://doi.org/10.3390/toxins15070458

Qin, B.; Deng, J.; Shi, K.; Wang, J.; Brookes, J.D.; Zhou, J.; Zhang, Y.; Zhu, G.; Paerl, H.W. and Wu, L. (2021). Extreme Climate Anomalies Enhancing Cyanobacterial Blooms in Eutrophic Lake Taihu, China. *Water Resources Research*, *57*(7), e2020WR029371. https://doi.org/10.1029/2020wr029371

Rodríguez, A.C.; Domínguez, C.O.; Puerto, M.; Cameán, A.M. and Jos, A. (2023). Immunomodulatory Effects of Cylindrospermopsin in Human T Cells and Monocytes. *Toxins*, *15*(4), 301. https://doi.org/10.3390/toxins15040301

Sabour, B.; Loudiki, M.; Oudra, B.; Vasconcelos, V.; Oubraim, S. and Fawzi, B. (2005). Dynamics and toxicity of *Anabaena aphanizomenoides* (Cyanobacteria) waterblooms in the shallow brackish Oued Mellah lake (Morocco). *Aquatic Ecosystem Health & Management*, *8*(1), 95–104. https://doi.org/10.1080/14634980590914944

Saoudi, A.; Brient, L.; Boucetta, S.; Ouzrout, R.; Bormans, M. and Bensouilah, M. (2017). Management of toxic cyanobacteria for drinking water production of the Ain Zada dam. *Environmental Monitoring and Assessment*, *189*, 361.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406-425.

Savadova-Ratkus, K.; Mazur-Marzec, H.; Karosienė, J.; Kasperovičienė, J.; Paškauskas, R.; Vitonytė, I. and Koreivienė, J. (2021). Interplay of Nutrients, Temperature, and Competition of Native and Alien Cyanobacteria Species Growth and Cyanotoxin Production in Temperate Lakes. *Toxins*, *13*(1), 23. https://doi.org/10.3390/toxins13010023

Stüken, A.; Campbell, R.; Quesada, A.; Sukenik, A.; Dadheech, P.K. and Wiedner, C. (2009). Genetic and morphologic characterization of four putative cylindrospermopsin producing species of the cyanobacterial genera *Anabaena* and *Aphanizomenon*. *Journal of Plankton Research*, *31*(5), 465–480. https://doi.org/10.1093/plankt/fbp011

Stüken, A.; Rücker, J.; Endrulat, T.; Preussel, K.; Hemm, M.; Nixdorf, B.; Karsten, U. and Wiedner, C. (2006). Distribution of three alien cyanobacterial species (Nostocales) in northeast Germany: *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. *Phycologia*, *45*(6), 696–703. https://doi.org/10.2216/05-58.1

Šuikaitė, I.; Vansevičiūtė, G. and Koreivienė, J. (2023). An overview of the distribution and ecology of the alien cyanobacteria species *Raphidiopsis raciborskii*, *Sphaerospermopsis aphanizomenoides*, and *Chrysosporum bergii* in Europe. *Oceanological and Hydrobiological Studies*, *52*(3), 312-326. https://doi.org/10.26881/oahs-2023.3.06

Sukenik, A.; Hadas, O.; Kaplan, A. and Quesada, A. (2012). Invasion of Nostocales (cyanobacteria) to Subtropical and Temperate Freshwater Lakes - Physiological, Regional, and Global Driving Forces. *Frontiers in Microbiology*, *3*, 86. https://doi.org/10.3389/fmicb.2012.00086

Touati, H.; Guellati, F.Z.; Arif, S. and Bensouilah, M. (2019). Dynamics of cyanobacteria in a Mediterranean reservoir of north-eastern Algeria: vertical and seasonal variability. *Journal of Ecology and Environment*, *20*, 93–107.

Usmonkulova, A.; Shonakhunov, A.T. and Kadirova, G. (2022). Activity of nitrogen-fixing cyanobacteria under salinity and heavy metals stress. *Journal of Pharmaceutical Negative Results*, *13*(3), 885-889. https://doi.org/10.47750/pnr.2022.13.03.055

Van de Waal, D.B.; Gsell, A.S.; Harris, T.D.; Paerl, H.W.; Senerpont Domis, L.N. and Huisman, J. (2023). Hot summers raise public awareness of toxic cyanobacterial blooms. *Water Research*, *242*, 120817. https://doi.org/10.1016/j.watres.2023.120817

Weralupitiya, C.; Wanigatunge, R.P.; Gunawardana, D.; Vithanage, M. and Magana-Arachchi, D. (2022). Cyanotoxins uptake and accumulation in crops: Phytotoxicity and implications on human health. *Toxicon*, *211*, 21–35. https://doi.org/10.1016/j.toxicon.2022.03.003

Wiedner, C.; Rücker, J.; Brüggemann, R. and Nixdorf, B. (2007). Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. *Oecologia*, *152*(3), 473–484. https://doi.org/10.1007/s00442-007-0683-5

Wu, C.-C.; Huang, W.-J. and Ji, B.-H. (2015). Degradation of cyanotoxin cylindrospermopsin by TiO2-assisted ozonation in water. *Journal of Environmental Science and Health, Part A*, *50*(11), 1116–1126. https://doi.org/10.1080/10934529.2015.1047664

Wu, Y.; Li, L.; Lin, L.; Zheng, L.; Dai, G.; Ma, H.; Shan, K.; Wu, H.; Zhou, Q. and Song, L. (2016). Patterns of succession between bloom-forming cyanobacteria *Aphanizomenon flos-aquae* and *Microcystis* and related environmental factors in large, shallow Dianchi Lake, China. *Hydrobiologia*, *765*(1), 1–13. https://doi.org/10.1007/s10750-015-2392-0

Zapomělová, E.; Hisem, D.; Řeháková, K.; Hrouzek, P.; Jezberová, J.; Korelusová, J. and Komárková, J. (2008). Experimental comparison of phenotypical plasticity and growth demands of two strains from the *Anabaena circinalis/A.*

crassa complex (cyanobacteria). *Journal of Plankton Research*, *30*(11), 1257–1269. https://doi.org/10.1093/plankt/fbn081

Zapomělová, E.; Jezberová, J.; Hrouzek, P.; Hisem, D.; Řeháková, K. and Komárková, J. (2009). Polyphasic characterization of three strains of *Anabaena reniformis* and *Aphanizomenon aphanizomenoides* (cyanobacteria) and their reclassification to *Sphaerospermum* gen. nov. (incl. *Anabaena kisseleviana*). *Journal of Phycology*, *45*(6), 1363–1373. https://doi.org/10.1111/j.1529-8817.2009.00758.x

Zapomělová, E.; Hrouzek, P.; Řezanka, T.; Jezberová, J.; Řeháková, K.; Hisem, D. and Komárková, J. (2011). Polyphasic characterization of *Dolichospermum* spp. And *Sphaerospermopsis* spp. (Nostocales, cyanobacteria): morphology, 16S rRNA gene sequences and fatty acid and secondary metabolite profiles. *Journal of Phycology*, *47*(5), 1152–1163. https://doi.org/10.1111/j.1529-8817.2011.01034.x

Zapomělová, E.; Skácelová, O.; Pumann, P.; Kopp, R. and Janeček, E. (2012). Biogeographically interesting planktonic Nostocales (Cyanobacteria) in the Czech Republic and their polyphasic evaluation resulting in taxonomic revisions of *Anabaena bergii* Ostenfeld 1908 (*Chrysosporum* gen. nov.) and *A. tenericaulis* Nygaard 1949 (*Dolichospermum tenericaule* comb. nova). *Hydrobiologia*, *698*(1), 353–365. https://doi.org/10.1007/s10750-012-1034-z