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RESEARCH ARTICLE

# Modern DNA Detection via Traditional Methods: Conforming Algal Species in Soran Independent Administration, Kurdistan Region of Iraq

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## ABSTRACT

This investigation was conducted on five genetically identified algal species at five sites in the Soran administration, with sampling monthly from March 2024 to January 2025. Accurate species identification is essential for understanding biodiversity, assessing ecosystems, and managing water quality. Traditional morphological identification is standard practice for algal characterization, but these methods are time-consuming and error-prone, especially when dealing with similar-looking species or mixed populations. Modern DNA detection methods, including polymerase chain reaction (PCR) and DNA barcoding, improve specificity and sensitivity. This study compares the effectiveness of DNA detection methods with traditional morphological techniques to confirm the presence of five algal species: Spirogyra fluviatilis, Rhizoclonium riparium, Paulschulzia pseudovolvox, Coelastrella tenuitheca, and Phaeocystis globosa, from approved algal checklists. This study evaluates the accuracy, reliability, and sensitivity of both approaches used to identify and screen these species in a specific area.

**Keywords:** Algae, DNA, Modern, PCR, Species

## Introduction

Identification of organisms using molecular techniques is faster and more accurate than traditional methods involving microscopy or culture techniques. These methods are preferred strategies for detecting organisms due to the growing awareness of species abundance, as well as the vast number of living microorganisms that cannot be isolated for survey in the lab.[1] Traditional morphological methods often fail to detect species and microorganisms, especially cryptic or poorly characterized taxa, necessitating molecular tools for precise identification. DNA-based approaches, such as COI barcoding, overcome these limitations by leveraging genetic divergence, enabling reliable discrimination of organisms across diverse groups—including plants, vertebrates, and highly variable viruses. This genomic strategy not only resolves taxonomic ambiguities but also provides a scalable, universal system for biodiversity assessment.[2] Identification and classification of species of algae utilizing specific antibody probes (involving monoclonal and polyclonal antibodies) and ribonucleic acid, along with quantitative Polymerase chain reaction (PCR) screening, tests are one of the newest Tools to consider for the benefit of traditional tools as much as possible to be utilized for quantification measurement.[3] The more effective and applicable method for identifying complex samples of living microorganisms is to use genes of ribosomal RNA, which contain small and large subunits. These genes have become the best benchmark tools in the field because they provide a more comprehensive point of view on population characteristics. Therefore, Sample formation can be easily obtained by cloning the DNA and then determining the nucleic acid composition by

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sequencing the targeted genes from samples taken from the organism.[4] Furthermore, ribosomal RNA databases are the most important instrument for identifying and discovering a large number of organisms. Specifically designed probes for the ribosomal RNA genes of specific species can be used to detect any combination of species and can be utilized in whole-cell techniques. Moreover, free of cells or morphological ways can also be used, where probes are applied directly to the DNA.[5] DNA-based ways, especially polymerase chain reaction (PCR) and barcoding DNA, have arisen as Strong tools for identifying species.[6] These molecular technologies display increasing privacy and sensitivity, and overcome the limitations of conventional methods by allowing species to be detected in pure and mixed populations. barcoding of DNA, which involves the utilization of a short marker of genetic (often from the 18S rRNA gene), has gained considerable attention as a reliable approach for algal identification of species.[7] Many studies have identified DNA from individual algal cells utilizing phylogenetic trees after culturing algal species collected from the environment or choosing samples of plankton that live in the aquatic system.[8,9] In this way, cells of algae are extracted within a small period (one to twenty-four hours) from samples originally collected under a light microscope utilizing capillary tubes. But the current is not always suitable, as it isolates unicellular algae with high skill requirements and taxonomic experience. This becomes a problem when they are combined, algae take longer to reach the identification stage in the laboratory, or when they are utilized for other purposes like analysis of genetic or molecular.[10] This problem encourages biologists to find the best ways to keep the cells of algae healthy and protect their DNA from damage so they can be used as a good model for molecular analyses. Therefore, molecular methods need an intact and healthy cell of algae to avoid any discrepancy in molecular data. and supply true identification of species of algae collected from the environment.[7] The aim of the current study is to discuss advanced molecular technologies that are utilized to identify different algae species at Soran University.

## Methods and material

### Description of the studied area

This studied area is located in the Soran independent administration in the Region of Kurdistan of Iraq, northeast of Erbil province, and falls within the category of cities with coordinates 36° 39′ 16.9092″ N and 44° 32′ 16.1052″ E[11,12] described the information related to the geology, climate, water resources, and soil conditions of the study area. Both studied sites (Gali 1 and Gali 2) are perennial springs located on the left side of the main road to Erbil. The area of Wadi Malakan covers different types of shallow springs, including Alana, Ble, and Malakan, which represent sites three, four, and five and are distributed in the mountain where Oak forests are located about 16 km south of Khalifan. The spring sites were determined by the global positioning system GPS and the results were expressed in X, Y, and Z systems, which were utilized in a software program (MapInfo) to draw a map of the study area Fig. 1 and Table 1.

### Algal collection for morphological identification

Samples of algae were put in vials, and one ml of Lugol solution was added to 100 ml of the sample collected from different springs water held until the identification of the morphology of the algae.[13,14] To retain the algae with its actual colour, a few drops of $CuSO_4$ were added to the sample.[15] In the current study, the identification of algae depended on the.[16]

### Polymerase chain reaction (PCR) amplification of ribosomal RNA genes (rRNA) for molecular identification of genus and species

#### *Molecular identification*

Algal samples were collected in 100-ml vials and were not preserved for molecular identification. Genomic DNA isolated from the algae was extracted using a Beta Bayern DNA extraction kit (Beta Bayern GmbH, 90453 Bayern, Germany).

#### *PCR Amplification*

Polymerase chain reaction (PCR) amplification of the 5.8S ribosomal RNA gene was performed in a total volume of 50 $\mu$l of a reaction mixture containing: Taq DNA polymerase master mix (AMPLIQON A/S Stenhuggervej 22), 10 pmol of ITS-F (TCCGTAGGTGAACCTGCGG) and ITS-R (TCCTCCGCTTATTGATATGC). DNase-free water and template DNA Table 2 using a Bioresearch PTC-200 gradient thermal cycler.[17,18] These primers were selected for their: (1) conserved binding sites across algal taxa, (2) consistent production of ~700 bp amplicons in validation tests, and (3) high-quality sequence matches in algal databases.

The temperature profile included step one, an initial denaturation at 95 C for 5 min, step two, followed by 40 cycles of a denaturation at 95 °C for 60 seconds, a primer annealing at 55 for 60 seconds, an extension at 72 °C for 1 min, and the final step is an extra extension at 72 °C for 8 min.

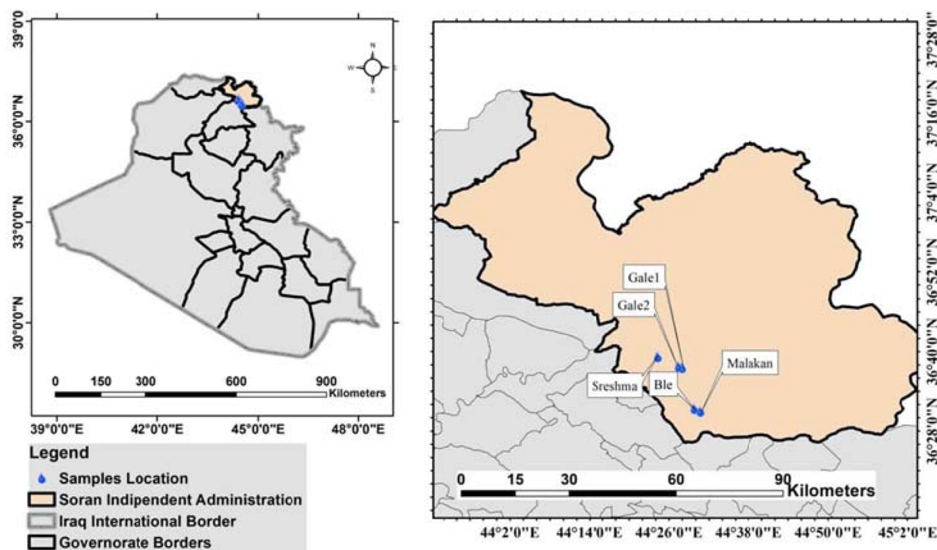


**Fig. 1.** A map of Iraq within Erbil Governorate; B Map of the study sites showing sampling sites (base map in ArcGIS).

**Table 1.** Sampling site location within the study area.

| Sites | Place | Type of habitat | X Field | Field | Z (Altitude) m.a.s.l |
|---|---|---|---|---|---|
| 1 | Gali 1 | Spring | 451333 | 4053896 | 855 m |
| 2 | Gali 2 | Spring | 451592 | 4053752 | 814 m |
| 3 | Sreshma | Spring | 450168 | 4044161 | 825 m |
| 4 | Bley | Spring | 450112 | 4044088 | 832 |
| 5 | Malakan | Spring | 450116 | 4044090 | 840 |

**Table 2.** PCR amplification reagents.

| No. | PCR components | Concentration | Volume ($\mu$l) |
|---|---|---|---|
| 1 | Master Mix | 2x | 25 |
| 2 | Forward Primer | 10 Pmol | 2 |
| 3 | Reverse Primer | 10 Pmol | 2 |
| 4 | DNase free Water | – | 18 |
| 5 | Template DNA | 50 ng/$\mu$l | 3 |
| | Total | | 50 |

### *Agarose gel electrophoresis*

DNA fragments were separated by electrophoresis through 1.2% agarose gels prepared in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3).[19] Briefly, agarose was dissolved by boiling and cooled to 50°C before adding ethidium bromide (0.5 $\mu$g/mL final concentration). Samples (5 $\mu$L) were mixed with 6× loading buffer (30% glycerol, 0.25% bromophenol blue) and electrophoresed at 100 V for 45 min alongside a 100–1500 bp DNA size marker (Thermo Scientific). DNA bands were visualized under UV illumination (Bio-Rad Gel Doc system).

### *DNA sequencing*

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced bidirectionally using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl DNA Analyzer (Applied Biosystems) at Macrogen Inc. (Seoul, Korea). Sequence chromatograms were analyzed and edited using FinchTV v1.4.0 (Geospiza).

### *Sequence analysis*

The 5.8S rRNA sequences were analyzed using BLASTn.[20] against the NCBI nucleotide database (accessed May 2024). Sequence alignments were performed using Clustal Omega.[21] with default parameters. Taxonomic identification was based on ≥98% sequence similarity to reference sequences in GenBank.

## Results and discussion

The stenothermal springs maintain relatively stable water temperatures and flow rates, and there is no anthropogenic effect, which provides a refuge for various aquatic organisms, including algae. Algal species composition and abundance in springs can differ significantly based on factors like temperature, elevation, nutrient availability, dissolved oxygen, hydrological condition, and others. The physical structure of springs influences algal distribution. Algae may occupy different niches, leading to varied

community structures.[22,23] However, features of morphology can lead to identification errors and might be more sensitive than molecular methods of identification. Samples of green algae were collected and categorized into five taxa by using a light microscope.[24] The classification of Spirogyra depends on its morphological properties, which can only be distinguished accurately taxonomically by a specially trained person. Furthermore, difficulties arise due to its small size and softness, the possession of some fixed morphological features, and its susceptibility to variations in phenotypic form. Consequently, the identification of more related Spirogyra species has relied solely on morphological properties, and as a result, confusion or misidentification may occur.[25,26] The most diverse genus is *Spirogyra*, which has six species occurring in Sreshma and Malakan springs. Another diverse genus is Coelastrella, with two species occurring in the Ble spring. During the months of April, June, and July, the growth of *Spirogyra fluviatilis* can reach its peak. The combination of high temperatures, abundant sunlight, and nutrient influx from runoff often results in extensive algal blooms. At the same time, the genus *Rhizoclonium riparium* was found in Gali 1 spring, which is a natural spring and shallow waters. It thrives in conditions with stable water flow and clear sunlight. *Coelastrella tenuitheca* thrives in such conditions, suggesting it has adapted to low-nutrient availability and potentially variable light conditions. Its thin cell wall may also play a role in its ability to withstand the environmental stressors, such as UV radiation or temperature fluctuation.[27] In this study, Paulschulzia pseudovolvox and Phaeocystis globose were both observed in Gali 2 spring during July and August. The variation in the different species present in the sites in this study may be related to the geology of the studied area, the site depths, seasonal variations, and conditions in the environment.[28] In conclusion, the evolutionary and identity systematics of Chlorophyta algae can be estimated molecularly sequence data. We have explained the feasibility of species-level identification, and polymerase chain reaction (PCR) analysis provides evolutionary data for these algae. Identification of the flowering Phaeocystis species in the studied area and a complete description of the various cell kinds are required to improve the basic knowledge about the taxonomy of *Phaeocystis*, biology, and ecology. To achieve this goal, we combined molecular (DNA-seq) analysis, microscopy (light microscopy), originating from a single strain of *Phaeocystis globosa* isolated from the Soran area.[29,30] We recorded Phaeocystis globosa at Site 2, where salinity averaged 7.5 ppt (peaking $>15$ ppt in dry seasons due to reduced freshwater inflow). Despite being below typical marine levels ($\sim$30–35 ppt), its presence likely reflects seasonal salinity shifts and local adaptation, as seen in North Sea populations.[31] Dry-season brackish conditions may enable survival via estuarine strain strategies.[32] Such as cyst formation or colony modulation. While P. globosa exhibits plasticity in low salinity (5–15 ppt), prolonged survival at $<10$ ppt remains uncertain.[33] Climate-driven salinity changes could expand its biogeographic range. The ITS primers utilized in this study have been validated for algal identification through previous research.[34,35]

It is obvious from Fig. 2 and Table 3 that the partial genes 5.8S rRNA sequences of six samples are aligned by the BLAST program from GenBank (http://blast.ncbi.nlm.nih.gov/) and were utilized to compare our amplified sequences with other GenBank stored sequences of species. The data from the BLAST indicated that the highest query sequence was 100% identical to various algae. The

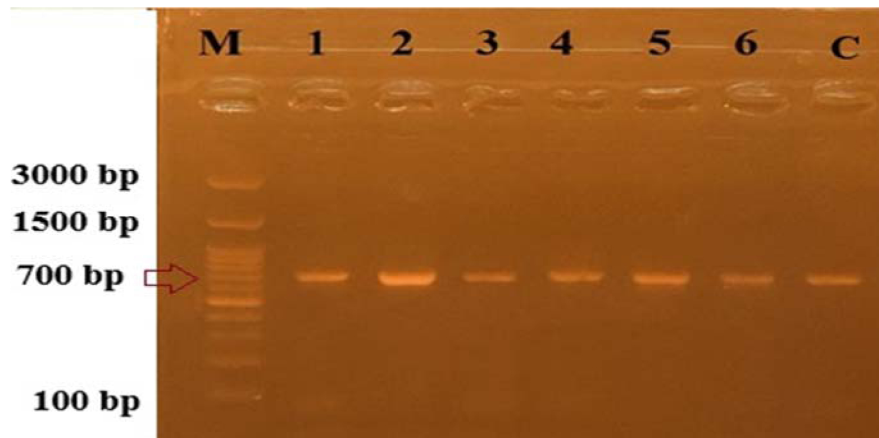


Fig. 2. PCR amplification of partial **5.8S rRNA** gene from six algae samples, in which the first lane is a marker ladder (3000 bp–100 bp), lanes 2–6 are 5.8S rRNA gene bands with a size of 700 bp, and the last lane C is a negative control without any band.

**Table 3.** Percentage distribution of samples of algae species according to blast of GenBank NCBI of the partial 5.8S gene.

| Algae samples Accession Number | Query Cover % | Identic Number % | GenBank Accession No. | GenBank algae Identification Species |
|---|---|---|---|---|
| PQ623464 | New record gene | | | Spirogyra fluviatilis |
| PQ623465 | 100 | 100 | JN399201 | Rhizoclonium riparium |
| | 100 | 99.5 | JN399202 | |
| PQ623466 | 100 | 100 | AF182428 | Paulschulzia pseudovolvox |
| PQ623467 | 100 | 100 | MH176121 | Coelastrella tenuitheca |
| | 100 | 100 | MH176122 | |
| | 100 | 99.49 | OR678637 | |
| PV016895 | 100 | 100 | MN603073 | Phaeocystis globose |

**Table 4.** Classification of algal taxa, new records, with their percentage in the study site, through the study period.

| Name of algal species | Genera | Species | % |
|---|---|---|---|
| Division: Chlorophyta<br>Class: Zygnematophyceae<br>Order: Zygnematales<br>Family: Zygnemataceae<br>Genus: *Spirogyra Link 1820*<br>Species: *Spirogyra fluviatilis* | 1 | 6 | 54 |
| Division: Chlorophyta (green algae)<br>Class: Ulvophyceae<br>Order: Cladophorales<br>Family: Cladophoraceae<br>Genus: *Rhizoclonium (Roth) Harvey, 1849*<br>Species: *Rhizoclonium. riparium* | 1 | 1 | 9 |
| Division: Chlorophyta (green algae)<br>Class: Chlorophyceae<br>Order: Volvocales<br>Family: Volvocaceae<br>Genus: *Paulschulzia* (P. Schulz) *Skuja 1948* Species: *Paulschulzia pseudovolvox* | 1 | 2 | 18 |
| Division: Chlorophyta<br>Class: Chlorophyceae<br>Order: Sphaeropleales<br>Family: Coelastreaceae<br>Genus: *Coelastrella Wang Q et al., 2019*<br>Species: *Coelastrella tenuitheca* | 1 | 1 | 10 |
| Division: Haptophyta<br>Class: Coccolithophyceae<br>Order: Phaeocystales<br>Family: phaeocystaceae<br>Genus: *Phaeocystis Scherffel, 1899*<br>Species: *Phaeocystis globosa* | 1 | 1 | 9 |
| Total | 5 | 11 | 100 |

recording of such new record species contributed to the habitat and nature of the study springs, Table 4.

## Description of the new records

### *Spirogyra fluviatilis hilse in Rabenhorst 1863*

Cells with plane end walls that are 30–45 um broad and 70–240 um long; three or four chloroplasts that make 1.5–3.5 cell rotations; zygospores ovoid, 47–85 um wide, 68–110 um long, median wall corrugate or finely wrinkled, brown; fertilized cells shortened, inflated, and up to 70 um broad; conjugation ladder-like, conjugation tubes created by both gametangia.[36] These species appeared in spring water and summer at site 5 (15 Apr 2024), Fig. 3A.

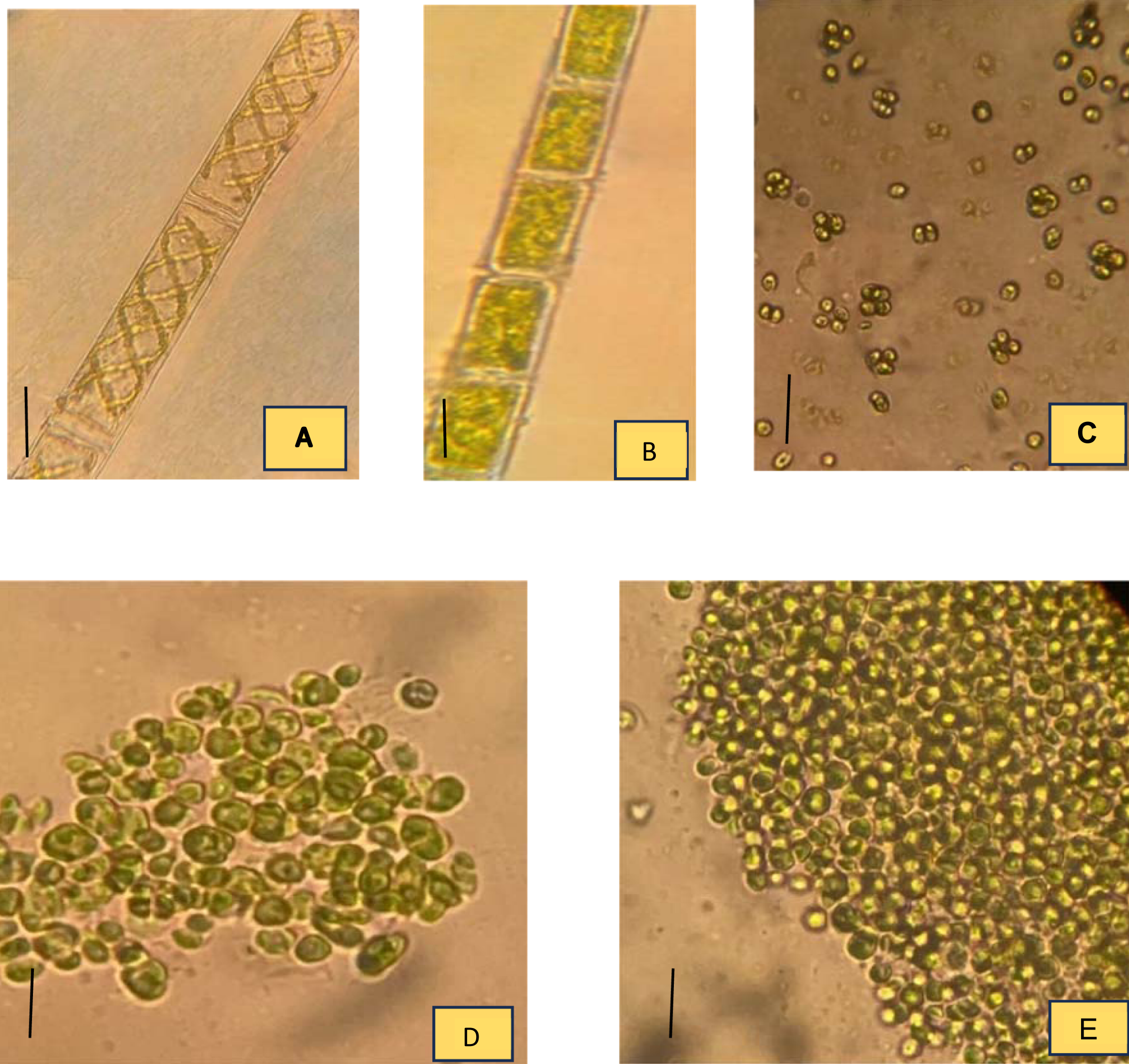


**Fig. 3.** Algal new record in the studied area: *A. Spirogyra fluviatilis, B. Rhizoclonium riparium, C. Paulschulzia pseudovolvox*, *D. Coelastrella tenuitheca E. Phaeocystis globosa* Scale bar: 10 $\mu$m.

### *Rhizoclonium riparium Kützing 1843*

Multinucleate, chloroplast net-like, uniseriate, filaments unbranched that occasionally have short, unicellular or multicellular branches rhizoidal; asexual reproduction through fragmentation, akinetes, and biflagellate zoospores; and sexual production through quadriflagellate gametes. Most common in flowing water, despite being primarily a marine species with a small number of occurrences, it is most likely worldwide. The only marine species in the British Isles is R. riparium (Roth) Harvey, which is found in brackish and marine water along the entire British Isles coast. It is particularly common on the upper shore and in salt marshes, and it may be able to survive for brief periods of time in completely freshwater habitats near the sea.[37] It was located in site 3 (15 Jun 2024), Fig. 3B.

### *Paulschulzia pseudovolvox (Schulz) Skuja 1948*

*Synonyms: Schulziella pseudovolvox (Schulz) teiling, tetra-spora nygaardii teiling*

Spherical or broadly ellipsoid colonies with 40–350 um in diameter and 2, 4, 8, 16, 32, 64 to 256 cells; in larger colonies, cells are frequently in sub-groups of 2–6 and encircled by an inner mucilage investment; cells are spherical, 6–13 um in diameter, thin-walled, and usually have two pseudocilia up to 200 um long, each of which extends past the mucilage envelope; chloroplasts are cup-shaped, occasionally lobed, and occasionally have a small eyespot. Germany; sporadically observed in the British Isles from lakes and ponds that are both moderately and severely nutrient-rich, either in the shallows and thereafter frequently linked to marginal aquatic vegetation (tychoplankton) or fully planktonic (euplankton).[38]

It occurs in the spring and has been recorded at site number 4. (15 May 2024) Fig. 3C.

*Coelastrella tenuitheca Qinghua Wang, Huiyin Song, Xudong Liu, Guoxing Liu & Zhengyu Hu*

Cells solitary or more rarely grouped, lemon-shaped, ellipsoid or broadly ovoid, thin-walled, bearing 6–12 (–18) often fine, spirally twisted ribs, some ribs meet apically where the wall frequently thickened; chloroplast parietal, initially single but later dividing into crowded fragments with each associated with a pyrenoid; reproduction asexually by 2, 4 or 8 (–16) fusiform or oval-shaped, autospores, arranged three-dimensionally in the mother cell and released by breaking the mother cell wall. Probably cosmopolitan; mostly on damp or wet terrestrial.[39] It is found in spring and was recorded at site 4 (15 May 2024), Fig. 3D.

*Phaeocystis globosa Scherffel, 1899*

The cell size of a tiny, unicellular phytoplankton species is usually between 5 and 20 micrometers. Both colonial and unicellular types are possible. The colonial form is made up of gelatinous masses that frequently show up on the water's surface as a greenish or yellowish froth. Has a greenish tint because of the presence of chlorophyll a and c; other pigments add to the color's overall complexity.[40] It was collected from site 2 (15 March 2024), Fig. 3E.

## Conclusions

Algal species detection through morphological examination remains an important means for recording and certification of the biodiversity of various algal species. Furthermore, molecular species detection, such as microscopic counting techniques, is still in its infancy. Algal species are characterized by large genomes with repetitive sequences and a remarkable ability to survive under the harsh conditions of their surrounding environment. These physical characteristics encourage scientists to explore and document algal biodiversity for various biological applications in biological assessments. Therefore, sequencing algal genomes will be a regular field of study in the near future to comprehensively map algal organisms.

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## Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Soran.

## Authors' contribution statement

F H A and J J T conceptualized and designed the study. F H A developed the methodology, performed the computational analysis, and supervised the research. J J T and B H R collected samples, conducted laboratory work, and performed species identification. All authors analyzed and interpreted the data, discussed the findings, and contributed to writing and revising the final manuscript.

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# الكشف الحديث عن الحمض النووي باستخدام الطرق التقليدية: توثيق أنواع الطحالب في إدارة سوران المستقلة، إقليم كردستان العراق

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**الملخص**

أُجريت هذه الدراسة على خمسة أنواع من الطحالب جرى تحديدها وراثيًا في خمس مواقع ضمن إدارة سوران، مع إجراء عينات شهرية للفترة من آذار 2024 إلى كانون الثاني 2025. يُعدّ التعرف الدقيق على الأنواع أمرًا أساسيًا لفهم التنوع الحيوي، وتقييم النظم البيئية، وإدارة جودة المياه. ويُعدّ التعرف المورفولوجي التقليدي ممارسةً معيارية في توصيف الطحالب، إلا أن هذه الطرق تستغرق وقتًا طويلًا وقد تكون عرضة للأخطاء، لا سيما عند التعامل مع أنواع متشابهة شكليًا أو تجمعات مختلطة. تُحسّن طرائق الكشف الحديثة المعتمدة على الحمض النووي، بما في ذلك تفاعل البوليميراز المتسلسل (PCR) وتقنية الباركود الجيني (DNA barcoding)، من مستوى الدقة والحساسية. تقارن هذه الدراسة فاعلية طرائق الكشف الجزيئي مع الأساليب المورفولوجية التقليدية لتأكيد وجود خمسة أنواع من الطحالب، هي Spirogyra fluviatilis :، و Rhizoclonium riparium، و Paulschulzia pseudovolvox، و Coelastrella tenuitheca، و Phaeocystis globosa، وذلك بالاستناد إلى قوائم الطحالب المعتمدة. كما تُقيِّم الدراسة دقة وموثوقية وحساسية كل من الطريقتين المستخدمتين في تشخيص هذه الأنواع وتحري وجودها في منطقة محددة.

**الكلمات المفتاحية:** الطحالب، الحمض النووي، حديث، PCR، الأنواع.