

# First record of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) in Türkiye

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## ABSTRACT

There are approximately 50000 species of algae. Algae contain economically valuable molecules, such as astaxanthin and polyunsaturated fatty acids (PUFA), and are used in food, cosmetics and animal feed industries. Green microalga *Haematococcus pluvialis* was considered to contain the highest amount of astaxanthin for many years. Recent research suggests that another green microalga, *Dysmorphococcus globosus*, contains more astaxanthin than *H. pluvialis*. *D. globosus* hasn't been studied extensively in the literature. In this study, approximately 1600 bp of the DNA barcoding gene *18S rRNA* was sequenced for identification of a new green alga from the Kaş district. Molecular identification and microscopy analysis show this new isolate belongs to genus *Dysmorphococcus*. This is the first record of genus *Dysmorphococcus* H. Takeda and *Dysmorphococcus globosus* H.C. Bold & Starr in freshwater algal flora in Türkiye.

## 1. Introduction

Algae are polyphyletic organisms which contain unicellular and multicellular species. They can be found in terrestrial, freshwater and marine environments. They are the primary producers in the marine ecosystem and produce 50-70% of oxygen on the planet. They have a great diversity in life cycles; many of them have a sexual cycle (Raven and Giordano 2014). Algae can be defined broadly as organisms which have oxygenic photosynthetic activity that is not higher plant. Currently, there are four kingdoms, fourteen phyla and sixty-three classes of algae. The fourteen phyla are, Cyanobacteria, Charophyta, Chromeridophyta, Glaucophyta, Rhodophyta, Chlorophyta, Euglenophyta, Dinoflagellata, Heterokontophyta (Ochrophyta), Picophyta, Haptophyta, Cryptophyta, Rhodelphiophyta, Prasinodermatophyta. There are 50589 species of living algae and 10556 fossil species. Chlorophyta (green algae) is the most diverse with eleven classes (Guiry 2024).

There are 5480 recorded algal species in Türkiye and genus *Dysmorphococcus* has not yet been recorded (Maraşlıoğlu and Gönülol 2025). The aim of this study was to isolate and characterize carotenoid containing algae. The *18S rRNA* sequence and cell morphology analysis show that the new isolated green algal strain belongs to the genus *Dysmorphococcus*. According to our database searches, the *Dysmorphococcus* species has not been recorded in Türkiye. We have reported the first isolation of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) from the Kaş district of Antalya.

## 2. Materials and Methods

### 2.1. Sample collection

The water sample was taken from a small rock pool that formed after rain water accumulation in the Kaş district of Antalya in February 2023 (Figure 1). The coordinates of the sample collection area are 36°21'24.28" N and 29°19'13.41" E and the elevation is 60 m. The color of the water was red, which suggested carotenoid containing algae could be present. The water sample was brought to the laboratory and examined under a microscope. Microscope observation showed that it contained several different microalgae as well as rotifer, which is known to feed on algae.

### 2.2. Growth conditions and isolation of the strain

A few milliliters of the water sample were inoculated into liquid tris acetate phosphate (TAP) medium (Gorman and Levine 1965) and kept under white light (approximately 40  $\mu\text{mol photons s}^{-1} \text{m}^{-2}$ ) at room temperature (20-25°C). The water sample was also spread onto solid TAP medium to allow colony formation. After visible colony formation, colonies were streaked several times to obtain axenic cultures. Single cells were separated under stereoscope with a flame drawn Pasteur pipette to obtain monoclonal cultures.



**Figure 1.** Location of the water sample collected for the isolation of the newly described *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu).

### 2.3. Morphological identification

The morphological species identification key prepared by Bold and Starr in 1953 was used for the identification of *Dysmorphococcus globosus* (Bold and Starr 1953). A Leica MC 190 HD light microscope was used for observation and to take photographs. Cells were observed using 40X objective lens and oil immersed 100X objective lens.

### 2.4. 18S rRNA gene sequencing

Primers 16S1N (5'-TCCTGCCAGTAGTCATATGC-3') and 16S2N (5'-TGATCCTTCT/CGCAGGTTTAC-3') were used to amplify 18S rRNA gene (Grzebyk et al. 1998). 2X GCTempase Mix (Amplicon, Denmark) was used in PCR reaction as recommended by the manufacturer. A total 35 cycles of PCR was used to amplify the gene. Annealing temperature was 55 °C and extension time was 2 minutes in each cycle. Agarose gel was used to visualize the product. The PCR product was purified and the sequence was determined by Sanger sequencing (BM Labosis, Ankara).

### 2.5. Molecular identification and phylogenetic analyses

The readings obtained from Sanger sequencing were used for molecular identification and phylogenetic relatedness. The forward read and the reverse read were trimmed from the end to remove the low-quality sequences, and then they were used to construct a more extended sequence of 1576 bp. This sequence was used to complete a search in the Basic Local Alignment Search Tool database (BLAST) from the National Center for Biotechnology Information (Zhang et al. 2000; Morgulis et al. 2008). Then phylogenetic analysis was performed by constructing a Maximum Likelihood phylogenetic tree, the tree was constructed using MEGA 12 (Kumar et al. 2024) with a bootstrapping value of 10000 replicates, and the tree was built using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions and the tree with the highest log-likelihood. The tree was rooted in *Chlorella vulgaris* (X13688) as an outgroup.

### 2.6. Scientific naming of the new taxon

The new taxon was named according to the literature (Takeda 1916; Bold and Starr 1953). "Instructions for Turkish Scientific Names of Plant, Fungi, Algae and Bacteria (Türkçe Bilimsel Bitki, Mantar, Suyosunu ve Bakteri Adları Yönergesi)" has been used for the naming of the new taxon (Menemen et al. 2021).

## 3. Results and Discussion

### 3.1. Morphology of the new isolate *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu)

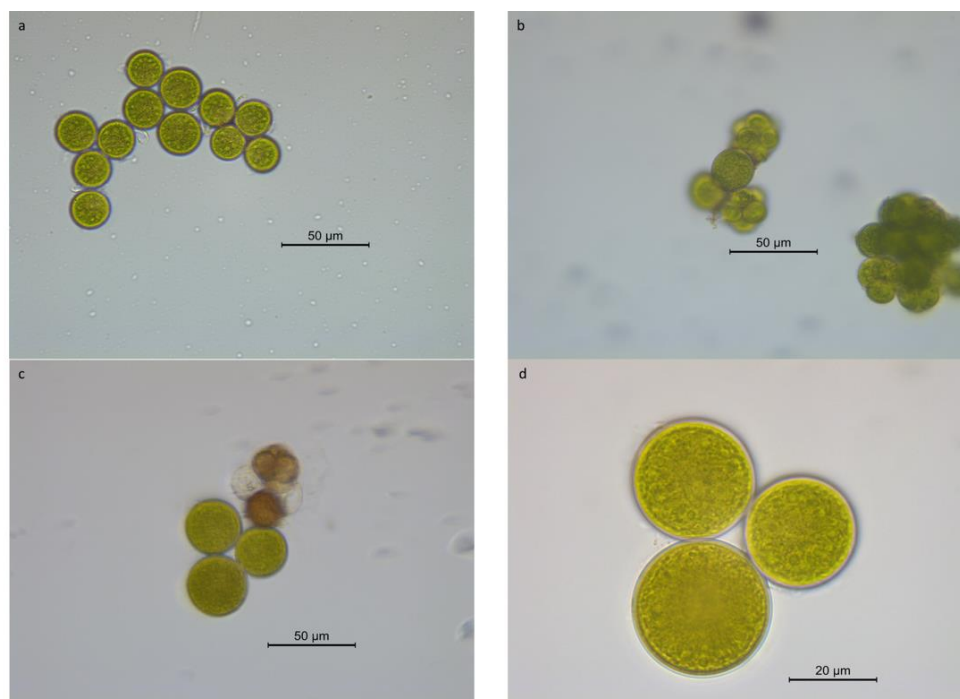
Light microscope images of the isolate are shown in Figure 2. The strain is very similar to morphology reported in the literature (Bold and Starr 1953; Jannel et al. 2023). Cells are round which is about 30 µm in diameter for fully grown cells; younger cells are smaller (Figure 2). As reported in the literature (Zohir et al. 2022) cells that are not stressed are green (Figure 2) and cells under stress have an orange color indicating accumulation of carotenoid astaxanthin (Figure 2c). Although the cells contain two flagella, motile cells were rarely seen in the observations, as also reported in the literature (Jannel et al. 2023). The Turkish name of this species is suggested as "Kınık su yosunu" according to the guidelines of Menemen et al. (2021).

### 3.2. Amplification of 18S rRNA gene and sequencing

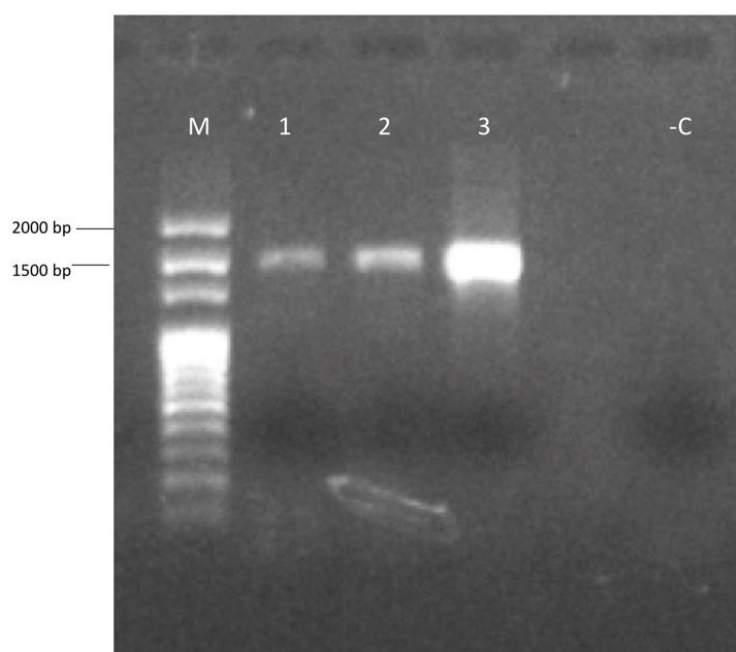
The expected product size for green algal species is about 1700 bp. As expected, a single specific band of about 1700 bp was formed in the PCR reaction (Figure 3). Green alga *Chlamydomonas reinhardtii* was used as a control in lane #3. The purified PCR product was sequenced using the same primers used for PCR in forward and reverse directions.

### 3.3. Molecular identification and phylogenetic analysis

The BLAST search results summarized in Table 1 shows that the isolate has 100% similarity with *Dysmorphococcus globosus*



**Figure 2.** Microscope images of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu). a) Green cells with 40X objective lens. b) Green cells that are dividing, 40X. c) Cells starting to accumulate carotenoid which have yellowish/orange color, 40X. d) Yellowish cells visualized using oil immersed 100X objective lens.



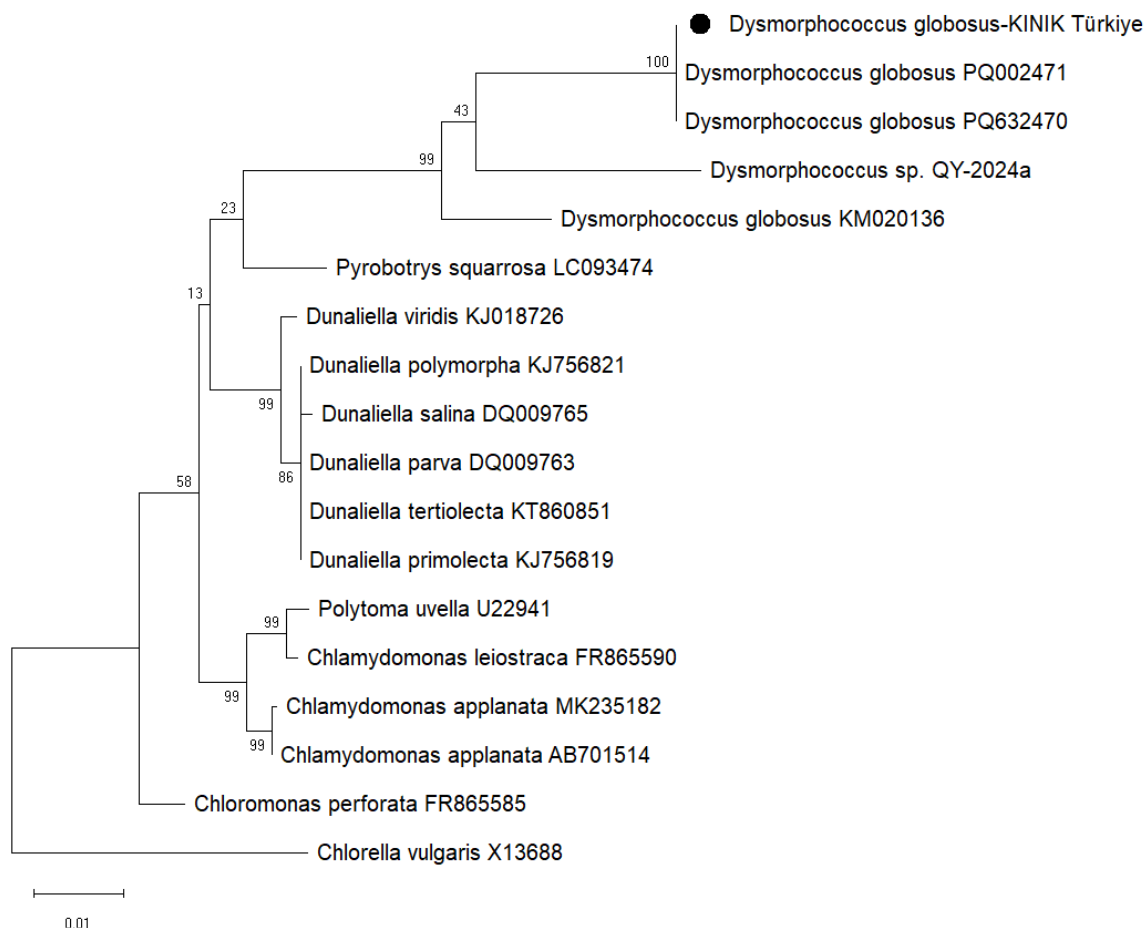
**Figure 3.** Amplification of 18S rRNA gene by PCR. A specific band of about 1700 bp was obtained. Line 1 and 2 are *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) product, line 3 is *Chlamydomonas reinhardtii* product. M; DNA size marker, -C; negative control.

**Table 1.** Summary of Blast search results

Sample	Scientific Name (Accession no.)	Max Score	Total score	Query Cover	E value	Percentage of identity
<i>Dysmorphococcus globosus</i> H.C. Bold & Starr (Kınık su yosunu) 1576 bp	<i>Dysmorphococcus globosus</i> (PQ632470.1)	2911	2911	100%	0	100
	<i>Dysmorphococcus globosus</i> (PQ002471.1)	2904	2904	100%	0	99.94
	<i>Dysmorphococcus globosus</i> SAG 20-1 (KM020136.1)	2556	2556	100%	0	96.18

(PQ632470.1) strain that was isolated from China, whilst it showed 96.18% similarity with the *Dysmorphococcus globosus* SAG 20-1 strain that is available at the Culture Collection of Algae at Göttingen University. This confirms that this isolate belongs to the *Dysmorphococcus* genus. Unfortunately, there are only a few sequences available for the 18S rRNA in the GenBank. In order to clarify the phylogenetic relation among these

sequences and others, a phylogenetic tree was constructed for these sequences in addition to other sequences from the related species that were obtained from culture collections worldwide (Figure 4). The results showed that the isolate that was obtained from Türkiye clustered with other isolates from the genus *Dysmorphococcus* as shown in Figure 4.



**Figure 4.** Maximum Likelihood phylogenetic tree constructed using the 18S rRNA. The bootstrapping value is 10000 replicates shown next to the branches, the tree is rooted to the outgroup *Chlorella vulgaris* (X13688).

### 3.4. Classification of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu)

Classification according to AlgaeBase database (Guiry and Guiry 2025).

Phylum: Chlorophyta  
Subphylum: Chlorophytina  
Class: Chlorophyceae  
Order: Chlamydomonadales  
Family: Phacotaceae  
Genus: *Dysmorphococcus*

Species: *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu)

This taxon occurred in rainwater accumulated in rock cavity formations in the antique city of Xanthos in the Kaş district of Antalya. This is a freshwater species.

## 4. Conclusion

There are 5480 records of algae in our country (Maraşlıoğlu and Gönülol 2025). Among these, 827 belong to Chlorophyta and with our new addition it reaches to 828 taxons. In this study, *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) which was isolated in Kınık village in the district of Kaş, Antalya has been provided as a new record for Turkish freshwater algal flora in light of molecular and morphological analyses.

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