Karadeniz Fen Bilimleri Dergisi, 15(1), 16-23, 2025. DOI: 10.31466/kfbd.1425464

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377 https://dergipark.org.tr/tr/pub/kfbd

Araştırma Makalesi / Research Article

Chlorolilaea pamvotia (Lortou&Gkelis) Lortou & Gkelis 2023: A new record of freshwater green algae (Chlorophyta) in Türkiye

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Abstract

Green algae (Chlorophyta) are photosynthetic organisms, the majority of which inhabit freshwater environments, with only a small proportion found in saltwater. This group represents a diverse range of species. Therefore, due to the morphological similarities and small size of different species, molecular characterization is essential for accurate identification. Molecular techniques, which have become increasingly widespread in recent years, allow for the identification of species that are morphologically indistinguishable or rare. In this study, 18S rRNA molecular sequencing was performed following the morphological identification of green algae isolated from Batlama Stream. The results confirmed that the isolated species was *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortu & Gkelis, 2023. This marks the first record of this species in the freshwater algal flora of Türkiye.

Keywords: Green algae, Freshwater, New record, 18S rRNA, Molecular characterization.

Chlorolilaea pamvotia (Lortou&Gkelis) Lortou & Gkelis 2023: Türkiye Tatlısu Yeşil Alglerinin Yeni Bir Kaydı

Öz

Yeşil algler (Chlorophyta), çoğunluğu tatlı sularda, çok az bir kısmı tuzlu sularda bulunan fotosentetik organizmalardır. Bu grup, çeşitlilik açısından zengin bir yelpazeye sahiptir. Bundan dolayı, farklı türlerin morfolojik benzerlikleri ve küçük boyutları nedeniyle doğru kimliklendirme için moleküler karakterizasyon gereklidir. Son yıllarda giderek daha yaygın hale gelen moleküler teknikler, morfolojik olarak ayırt edilemeyen veya nadir görülen türlerin kimliklendirilmesine olanak tanır. Bu çalışmada, Batlama Deresi'nden izole edilen yeşil alglerin morfolojik kimliklendirmesinin ardından, 18S rRNA moleküler dizileme yöntemi uygulanmıştır. Elde edilen sonuçlar, izole edilen türün *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortu & Gkelis, 2023 olduğunu ve bu türün Türkiye'nin tatlı su alg florasında ilk kez kaydedildiğini ortaya koymuştur.

Anahtar Kelimeler: Yeşil alg, Tatlı su, Yeni kayıt, 18S rRNA, Moleküler karakterizasyon.

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Geliş/Received: 25.01.2024 **Kabul/Accepted:** 18.12.2024 **Yayın/Published:** 15.03.2025

Altürk Karaca, S., Soylu, E. N. (2025). *Chlorolilaea pamvotia (Lortou&Gkelis)* Lortou & Gkelis 2023: A new record of freshwater green algae (Chlorophyta) in Türkiye. *Karadeniz Fen Bilimleri Dergisi*, 15(1), 16-23.

1. Introduction

Algae are photosynthetic organisms, either prokaryotic or eukaryotic, that lack true organ differentiation, such as roots, stems, or leaves. They possess the ability to transform environmental nitrogen and phosphorus into biomass through the utilization of light, CO_2, and water (Ometto et al., 2014). As one of the most prevalent groups in aquatic ecosystems, algae thrive in a wide array of habitats, including freshwater lakes, low- and high-salinity waters, seas, glaciers, deserts, and hot springs (Agger et al., 2008). In these environments, algae occupy two primary niches: benthic (attached) and phytoplanktonic (free-floating). Benthic algae can be epiphytic (growing on plants), epilithic (attached to stones or hard substrates), epizoic (on animals), or epipelic (on sediment and sand).

Microalgae serve as primary producers in both freshwater and marine ecosystems. Typically measuring between 2 to 50 micrometers, microalgae exhibit a diverse array of morphological forms, existing as either unicellular or multicellular organisms (Bermejo et al., 2021). Major groups of microalgae include green algae (Chlorophyta), blue-green algae (Cyanobacteria), golden-yellow algae (Ochrophyta, Xanthophyta), diatoms (Bacillariophyta), red algae (Rhodophyta), and brown algae (Phaeophyta), alongside other groups such as Dinophyta, Haptophyta, Chrysophyta, Eustigmatophyta, Raphidophyta, Cryptophyta, and Glaucophyta (Lee et al., 2020; Smith et al., 2021).

Among microalgae, green algae (Chlorophyta) represent a particularly diverse group, with members ranging from microscopic single-celled species to large spherical colonies and extensive filamentous forms in freshwater environments. Their characteristic green coloration, resulting from chlorophyll-a and -b, distinguishes them from other algal groups. With over 17,000 recognized species, green algae constitute the most species-rich division within the algal lineage (Graham and Wilcox, 2000). Their morphological diversity—spanning unicellular, colonial, and filamentous forms—demonstrates the broad spectrum of developmental strategies within this group.

Precise identification and nomenclature of microalgae are foundational to research, analysis, and experimental studies. Correct taxonomic classification is crucial for ensuring reproducibility, repeatability, and accuracy across various biological disciplines (Bortolus, 2008). While light microscopy has traditionally been the primary method for identifying microalgal species, it has notable limitations. Small microorganisms and morphologically similar taxa, such as spherical green algae, can be challenging to distinguish under light microscopy alone (Krienitz and Bock, 2012). Additionally, certain life cycle stages may obscure taxonomic identification (Rowan and Powes, 1991).

In recent years, molecular techniques have become invaluable for the identification of morphologically similar or cryptic species (Soylu and Gönülol, 2012). These approaches are

particularly effective for identifying rare species and those that cannot be readily cultured (Forney, 2004; Dorigo, 2005; Tringe, 2008). Currently, ribosomal RNA gene sequencing is widely employed in microbial ecology to elucidate the diversity and composition of microbial communities. For prokaryotes, the 16S rRNA gene is commonly targeted, while the 18S rRNA gene is the preferred marker for eukaryotic diversity studies (Fawley and Fawley, 2004). The 18S rRNA gene, with its conserved regions and repeating sequence motifs, serves as an ideal tool for phylogenetic analysis and microalgal identification (Khaw et al., 2020). In this study, green algae isolated from Batlama Stream were identified both morphologically and molecularly through analysis of the 18S rRNA gene sequence.

2. Materials and Methods

2.1. Microalgae Isolation, Purification and Cultivation

Water samples were collected from pelagic (surface) zone of Batlama Stream. These samples were transported to the laboratory in sterile 1 L plastic bottles under cold conditions to preserve their integrity. A 1 ml aliquot of each sample was inoculated onto BG11 agar plates solidified with 1% agar. The plates were incubated at a temperature of 20°C with a 12:12 light/dark cycle under a light intensity of 155 µmol/m²/s. After one month, distinct colonies were observed, which were isolated using a sterile inoculation loop. These isolated colonies were further transferred to solid media until a single species was identified (Demiriz, 2008). The isolated species were transferred to liquid media and left to grow in the climate cabinet.

Figure 1. Location of the newly described species

2.2. Morphological Identification

Samples were collected from species growing in liquid media, maintaining sterile conditions within a laminar flow cabinet to avoid contamination. The sizes of the samples were determined using light microscopy and inverted microscopy with the aid of an ocular micrometer. Taxonomic identification was performed following the classification provided by Lortou et al. (2022). The nomenclature currently accepted in the scientific community was verified through comparison with AlgaeBase and the Turkish algae database (Guiry and Guiry, 2023; Maraşlıoğlu and Gönülol, 2023).

2.3. Molecular Identification

At the end of the incubation period, a 1800 base pair fragment of the 18S ribosomal RNA gene was amplified using PCR. DNA sequence analysis was subsequently performed, and the obtained sequence data was compared with sequences in sequence databases containing validated microbial sequences. Based on this comparison, the group to which the microalgae belonged was determined. The DNA sequencing analyses were conducted at BM Software Consulting and Laboratory Systems Limited Company.

3.Findings and Discussion

In the samples sent to BM Software Consulting and Laboratory Systems Limited Company, the identification of microalgae was conducted through polymerase chain reaction (PCR) amplification targeting an 1800 base pair fragment of the 18S ribosomal RNA (rRNA) gene, which is commonly used for the molecular identification of microalgal species. The PCR amplification was followed by DNA sequencing, allowing for the acquisition of a detailed nucleotide sequence from the amplified region of the gene. This sequence was then subjected to comparison with a range of entries in specialized microbial sequence databases, which contains extensive collections of validated genetic data from known microbial species.The sequence comparison revealed that the isolated microalgal species exhibited a 97.39% sequence similarity to *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortou & Gkelis, a species that was first described by Lortou et al. (2022). This species was originally isolated from Lake Pamvotis, a freshwater lake located in the Mediterranean region. *Chlorolilaea pamvotia* was noted for its contribution to the biodiversity of photosynthetic microorganisms in this area, enriching the knowledge of algal diversity in Mediterranean freshwater ecosystems. The identification of this species in Türkiye represents a significant extension of its known distribution, marking the first record of *Chlorolilaea pamvotia* in the freshwater algal flora of the country. The

genetic evidence obtained from this study further substantiates its identification as *Chlorolilaea pamvotia*, based on the high degree of sequence similarity to the reference data.

3.1. Classification of *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortou & Gkelis,2023

Phylum: Chlorophyta
Subphylum: Chlorophytina
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Chlamydomonadales incertae sedis
Genus: Chlorolilaea
Species: *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortou & Gkelis,2023
Basionym: *Lilaea pamvotia* Lortou & Gkelis

Description: The vegetative cells exhibit a spherical to irregular shape, measuring 7–16 μm. These cells tend to form aggregates without mucilage and occasionally arrange into colonies with randomly distributed cells. The chloroplasts are cup-shaped to reticulate, featuring thylakoids organized into bundles of varying thickness. Some chloroplasts may contain an eyespot and one or several pyrenoids surrounded by starch plates. Each cell contains a single nucleus. In mature cultures, significant accumulations of starch and plastoglobuli (lipid droplets) are found in both the chloroplasts and cytoplasmic oil bodies. The thylakoids are organized into bundles of different sizes and thicknesses. The organism undergoes asexual reproduction via aplanospores (Figure 2). *Chlorolilaea pamvotia* species phylogenetic tree is presented in Figure 3 (Lortou et al., 2022).

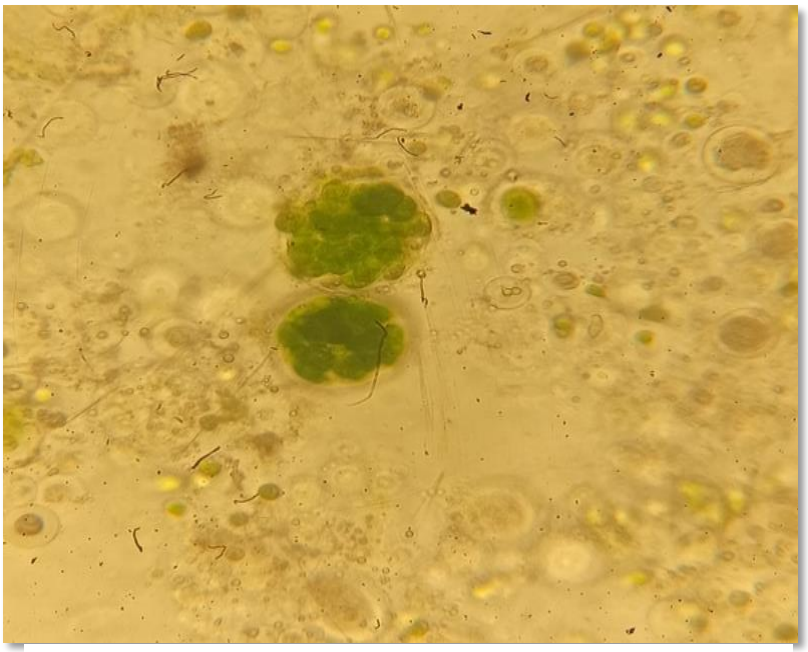

Figure 2. Light micrograph of strain *Chlorolilaea pamvotia*

Type Locality: This taxon occurred as planktic in the water column of Lake Pamvotis (39◦40′51″ N, 20◦50′30″ E) and was collected on 1 November 2010 from a surface layer (0–0.5 m) at inshore sites.

General Environment: This species is freshwater-specific.

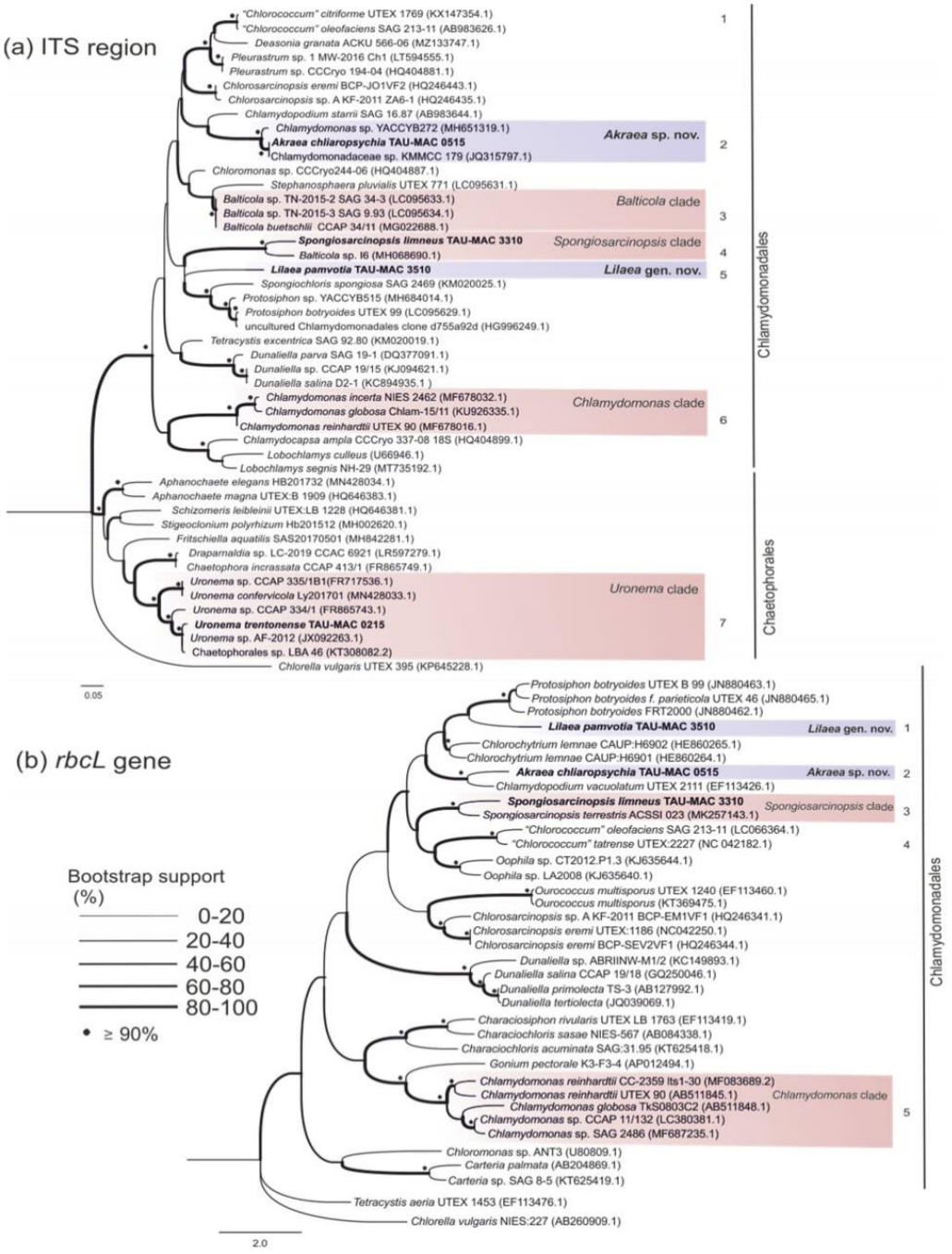

Figure 3. Phylogenetic tree of *Chlorolilaea pamvotia* (Lortou et al., 2022).

4. Conclusion and Recommendations

The utilization of a partial sequence of the 18S rRNA gene is commonly embraced and widely employed method for identifying microalgae (Moon-van der Staay, 2001; Friedl, 2002; Krienitz, 2011; Leliaert, 2014). As a result, based on the obtained morphological and molecular data, it has been determined that the species isolated from Batlama Stream is *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortou & Gkelis,2023. This species was previously isolated from Lake Pamvotis and described by Lortou and colleagues (Lortou et al., 2022). This study is important as it represents the first record of this species in Turkish waters. This result is believed to contribute to the algal floras of Türkiye.

Acknowledgements

The authors would like to thank BM Software Consulting and Laboratory Systems Limited Company for their contribution to this research.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

The authors declare that there is no conflict of interest.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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