First record of *Coelastrella vacuolata* (Chlorophyta: Scenedesmaceae) in Tuticorin coast, Gulf of Mannar

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Present study was aimed to isolate marine green algae from the sea water samples of Tuticorin coast, Gulf of Mannar. The collected seawater was maintained in Walne's medium with optimum conditions (pH: 8.2-8.7, light intensity: 1000 Lux, salinity: 35 ppt and temperature: 18-25 °C and) for obtaining maximum growth. Then the mixed culture was purified by streak plate method and monoalgal sample was isolated. Monoalgal sample was identified as *Coelastrella vacuolata* by Light Microscope (LM), Fluorescent Microscope (FM) and Scanning Electron Microscope (SEM) based on its morphology. It was further confirmed by the molecular identification by 18s rDNA sequencing. Cells were observed either in isolated or in the colony and the size was ranged from 7 to 11 μ. It is the first record for the presence of *C. vacuolata* in the seawater of Tuticorin coast, Gulf of Mannar.

[**Keywords**: Algae; *Coelastrella vacuolata*; Seawater]

Introduction

Gulf of Mannar is the only and the most important marine Biosphere Reserve in India which was first recognized in the year of 1980. In total, 21 islands are present in geographical area surrounded by coral reef with shallow water habitat of unique marine biodiversity. It contains diversity of marine plants and animals. The Gulf of Mannar region is the shelter for 3600 species of flora and fauna. Remaining flora and fauna are yet to be identified. Therefore, identification and documentation of organism present in Gulf of Mannar region is very important[1].

Algae are an important class of marine living organisms used for various applications. In recent times, algal diversity have been demonstrated based on morphological and molecular information[2] and they have been arranged in six different green algal classes[3]. Phylogenetic analyses of algae revealed that multiple different terrestrial organisms have evolved from aquatic ancestors[4].

Particularly, the family, Scenedesmaceae have been studied for genetic and physiological controls over cell division by multiple fission[5], to find out evolution of terrestrials within green plants[2,3], and for the water quality parameters in any aquatic system[6].

Recent decade, attention on microalgae increased due to potential biomolecules they produce[7]. Moreover, it is able to efficiently fix carbon dioxide through photosynthesis, which may help in mitigating global climate changes caused by pollutions. Microalgae produce a variety of primary metabolites such as proteins, starch, cellulose and lipids as wells as secondary metabolites including pigments, flavonoids, phenolic compounds and potential pharmaceuticals[7].

In this context, the aim of the present investigation was to isolate and identify the particular algal strain from Tuticorin coast, Gulf of Mannar.

Materials and methods

Study area

Seawater samples were collected for the isolation of marine microalgae from the Tuticorin Coast, Gulf of Mannar with the Latitude of 8° 78' N and Longtitude of 78° 16' E.

Collection and maintenance of seawater sample

Seawater samples were collected in sterile glass containers and were stored at 18-25 °C until use. The water samples containing microalgae were inoculated and maintained in Walne's medium in

conical flasks at 18-25 °C, salinity of 27-35 ppt with white fluorescent light at an intensity of 1000 lux. Growth of mixed microalgae was observed regularly by light microscope.

Isolation and identification of micro algae

Initially, the aliquots from the mixed species were collected and subjected to compound microscope examination. The wet mounts of the species were taxonomically determined using identification manual[8]. Then the aliquots of mixed species were subjected to serial dilution followed by the quadrant streaking for pure cultures. Stock cultures of pure cultures were maintained in a special room adjacent to the mass culture and maintained in Walne's medium and the growth potentials were assesed at regular spectral course. Microscopic identification of the algal species was performed under different magnifications in Light Microscope (LM) and Fluorescent Microscope (FM).

Scanning electron microscope (SEM)

For scanning electron microscopy (SEM), algal cells were dehydrated in gradually increasing ethanol concentrations (up to 96 % ethanol), transferred in formaldehyde-dimethyl-acetal (FDA, dimetoxymethane for 24 hours and 2 hours, critical-point dried with CO_2, sputter-coated with palladium/gold and examined with a VEGA 3 TESCAN SEM microscope.

Identification of marine microalgae through 18S rRNA

The algal genomic DNA was isolated using Himedia plant DNA extraction kit according to the manufacturer's instructions. The cultured microalgae were identified through 18S rRNA by their specific primers designed based on the available sources from NCBI for different marine microalgal strains (ITS Primer- Sequences 5' – 3': Forward- TCCGTA GGTGAACCTGCGG and Reverse-TCCTCCGCTT ATTGATATGC). The PCR amplification was performed with the initial denaturation for 4 mins at 94 ºC followed by 35 cycles of denaturation for 30 sec at 94 ºC, annealing for 30 sec at 55 ºC and extension for 30 sec at 72 ºC followed by final extension for 10 mins at 72 ºC. The amplified fragment was gel eluted and analyzed by sequencing and submitted in GenBank for species identification. Sequences from various *Coelastrella* sp. and related groups were aligned using ClustalW. Neighbor-joining nd maximum-likelihood trees were built using MEGA[9].

Results

Microscopic observation

Based on the morphology obtained, the isolated species was identified as *Coelastrella vacuolata* (Fig. 1). Figure 2a shows the clear visible vacuole present inside the cell which is the key identification character for *C. vacuolata.* It was further confirmed by the key model published by Skaloud[10] (Fig. 2b). The classification of *C. vacuolata* is given below:

Empire:	Eukaryota
Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrakingdom:	Chlorophyta
Phylum:	Chlorophyta
Subphylum:	Chlorophytina
Class:	Chlorophyceae
Order:	Sphaeropleales
Family:	Scenedesmaceae
Subfamily:	Coelastroideae
Genus:	*Coelastrella*
Species:	*vacuolata*

Description: Cells 7 to 11 µm long solitary coccoid shaped; asexual reproduction by spindle shaped or broadly coccus; autospore arranged three
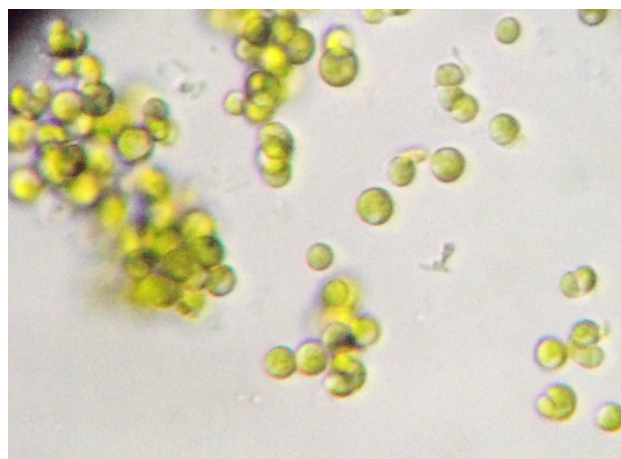

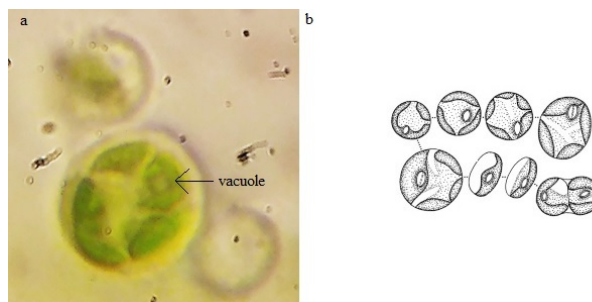Fig. 1 — Microscopic observation of *C. vacuolata* in 45X.

Fig. 2 — (a) Microscopic observation of *C. vacuolata* in 100X, (b) Key model for identification of *C. vacuolata*[10].

dimensionally in mother cell and released by fracture of mother cell wall. They are appeared in single cells or colony.

Figure 3 shows *C. vacuolata* under fluorescence microscope and the red fluorescence is from the chlorophyll in the chloroplast of *C. vacuolata*.

SEM

Figure 4a shows the SEM image of *C. vacuolata* and it clearly shows the coccoid structures without any ribs and granules on cell wall. Figure 4b shows autosporangia with autospore.

Molecular Identification

A total of 558 base pairs sequence was obtained from 18s rRNA sequencing (Fig. 5) and submitted to Genbank (Accession number MH243551).

Fig. 3 — Observation of *C. vacuolata* under fluorescence microscope.

The maximum-likelihood phylogenetic tree is showed in Fig. 6. *Coelastrella vacuolata* is labelled as MH243551 and it has maximum similarity to the sequence of >gi|KY426801 and thus the two share a common ancestor. Closest sequence is >gi|KU744518 with 98 % similarity. The overall tree helps us to understand the similarity of 18s rDNA sequence of *C. vacuolata* to other DNA sequences of *Coelastrella* sp. in the database of NCBI and also confirms the identity of the organism.

Discussion

In earlier studies, the family Scenedesmaceae has been studied in broad way which included many species with different morphology[10]. HortoBágyi[11] and Skaloud[10] identified many taxa using light microscopic studies. Similarly, *C. vacuolata* was identified by clear visible vacuole present inside the cell with the help of light microscope.

Regular changes in genera or taxa from the Scenedesmaceae family, description of genera and species in that family became more difficult. The species of this family has been characterized by a unique outer cell wall ultrastructure[12]. In early 90's

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TGGGTACTACCCGGTCGTACTCACAGCTGGGTGGGCATTGTTGCCTGCTCAGTGGCGCCTTGGCATGATCATA
CACCAGTGCTAACCACTGATAAAACCAAACTCTGAAGTTTGATTGCTATTAACTGGCAATCTTAACCAAAGACA
ACTCTCAACAACGGATATCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTG
CAGAATTCCGTGAACCATCGAATCTTTGAACGCATATTGCGCTCGAGCCTTCGGGCAAGAGCATGTCTGCCTC
AGCGTCGGTTTACACCCTCACCCCTCCCTTTCTTGGGTGTGTTGATCTTTGATCAACCATTGGGGTGGATCTGG
CTTCCCCAATCTGCCTTGTAGCGGATTGGGTTGGCTGAAGCACAGAGGCTTAAGCAAGGACCCGATATGGGCT
TCAACTGGATAGGTAGCAACGGCTTGTGCCGACTACACGAAGTTGTTGCCTGTGGACTTTGCTAGAGGCCAAG
CAGGAACATGCTTTATGCATGCCTAAACTTTCGACCTGAGCTCAG
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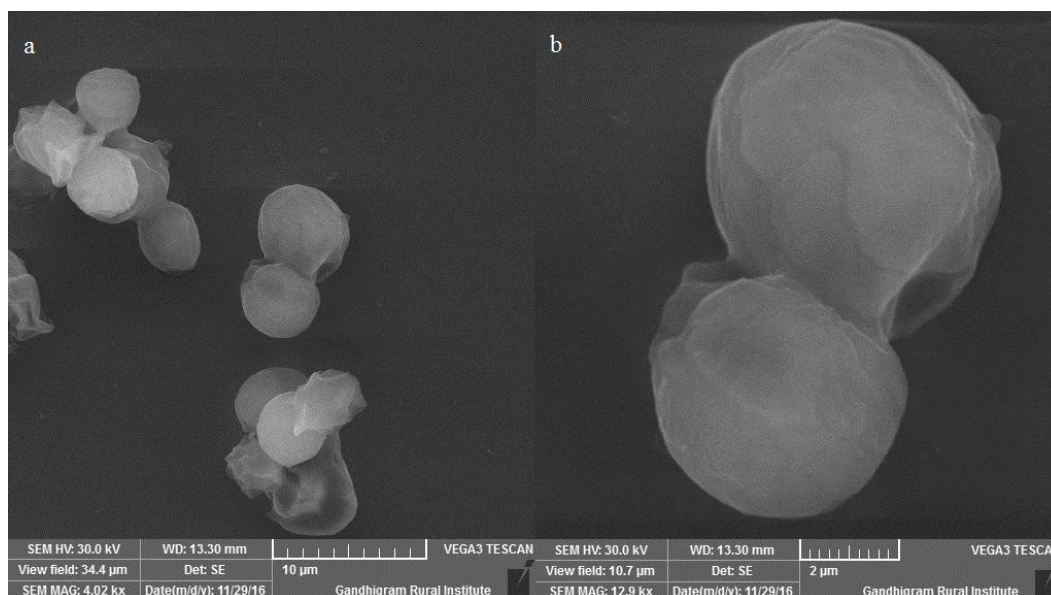Fig. 5 — 18s rRNA sequence of the amplified fragment using ITS primers.

Fig. 4 — SEM image of *C. vacuolata* (a) group of cells without ribs and granules on cell wall (b) autosporangia with autospore.
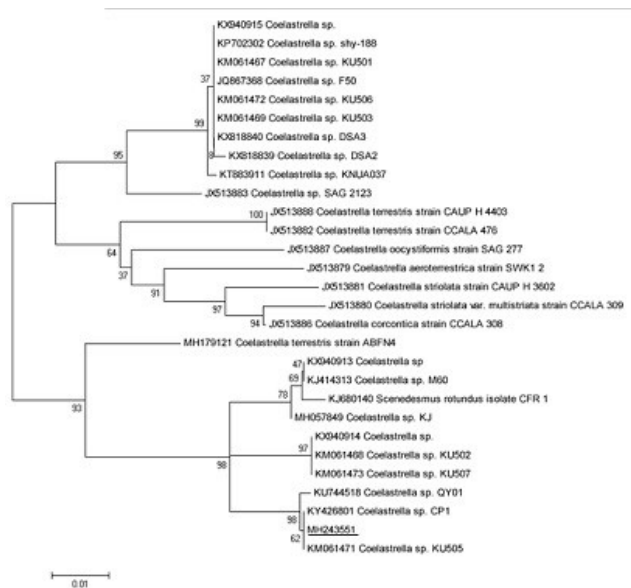
Fig. 6 — A Phylogenetic tree of randomly selected sequences from the results of BLAST were aligned using MEGA 6, ClustalW standard parameters.

variations in morphology was further evaluated by electron microscopic studies[13]. In this study, SEM image of *C. vacuolata* clearly shows the coccoid structures without any ribs and granules on cell wall. Moreover, granulation is formed due to the precipitation and it is one of the key factors for identification which lack in *C. vacuolata*[14].

The demarcation of genera and species in that family became more difficult, and molecular phylogenetic studies revealed more complexity because morphological and molecular identification were not always similar[15]. Therefore, *C. vacuolata* was subjected to molecular identification and it showed maximum similarity to the sequence of KY426801. The overall phylogenetic tree helped us to understand the similarity of 18s rDNA sequence of *C. vacuolata* to other DNA sequences of *Coelastrella* sp. in the database of NCBI.

Conclusion

Till date, there is no clear report regarding the biodiversity of Scenedesmaceae family in Tuticorin coast, Gulf of Mannar. In this context, *C. vacuolata* was isolated for better understanding of the taxonomy and diversity and it is the first record from Tuticorin coast, Gulf of Mannar region.

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