

THE MORPHOLOGY, SPORES FORMATION AND GERMINATION, LOCAL DISTRIBUTION OF *CLADOPHORA FLEXUOSA* (O.F. MULLER) KUTZING AND *CLADOPHORA PROLIFERA* (ROTH) KUTZING FROM MYANMAR

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Abstract

Two species of genus *Cladophora* belonging to the Cladophorales, Chlorophyta collected from the three Coastal Regions of Myanmar in 2009 to 2013 were identified as *C. flexuosa* (O.F. Muller) Kutzing and *C. prolifera* (Roth) Kutzing. The descriptions of each taxon with emphasis on the shape, size, colour and branching type of cell and rhizoid. Culture study on spores formation, germination on two species of the genus *Cladophora*. In addition, the distribution ranges of each species along the three coastal regions of Myanmar were presented.

Keywords: *Cladophora*, morphology, laboratory culture, local distribution.

Introduction

Marine algae are the large primary producers of the sea: during the process of photosynthesis inorganic carbon (CO₂) is transformed into organic compounds using radiation energy from the sun and with the simultaneous release of oxygen. Algae were probably the first photosynthetic organisms and untitled appearance of plants on earth, the only photosynthesizers for billions of year. This process is the basis for all plant and animal life. Marine algal beds provide feeding, spawning and nursery grounds for marine living organisms (Chin *et.al.*, 2015).

There are 1061 species names in the genus *Cladophora*, of which 188 have been flagged as currently accepted taxonomically (Guiry, 2013). In 2010, Soe-Htun reported the current status of marine algae with a total of 111

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genera and 229 species growing along the three Coastal Regions of Myanmar. Kyaw Soe and Kyi Win (1977) reported the occurrence of twelve species of *Cladophora* along the Coastal Regions of Myanmar. Moreover, Soe-Htun *et.al.* (2009) accounted the five species of *Cladophora*, viz., *C. prolifera* (Roth) Kutzing, *C. rupestris* (Linnaeus) Kutzing, *C. vagabunda* (Linnaeus) Hoek, *C. saracenic*a Boergesen and *C. sibogae* Reinbold along the three Coastal Regions of Myanmar.

The purposes of this study are: 1) to identify the two species of *Cladophora* Kutzing, 2) to study the reproductive structures, 3) to record the distributional ranges of two species of *Cladophora* Kutzing both along the three Coastal Regions of Myanmar.

Materials and Methods

The samples were collected from intertidal zone, along the three Coastal Regions of Myanmar from 2009 to 2013. Fresh and living plants were collected from several localities and saved in ice box and brought to the laboratory of Mawlamyine University for observation. Fresh and living plants of specimens were fixed and preserved in 5 % formaldehyde solution which was prepared with seawater. Some of these were prepared for herbarium specimens. All voucher materials in the forms of wet-stack or herbarium specimens were deposited at the Herbarium of Department of Marine Science, Mawlamyine University, Myanmar (MMB). Liquid-preserved specimens were used for detail investigations on external morphology and habit of the plants. Cultures were kept in freezer-incubators illuminated with cool white fluorescent lamps (100-200 fc). Culture Petridishes were incubated under 16 light : 8 dark photoperiod in incubators and in room temperature. Early development of sporelings was examined and diameter (in length) of sporangia was measured under electron microscope with the help of ocular meter of 5 days intervals. Culture medium was changed after each examination.

Results

The two species of *Cladophora* Kützing were collected from the three Coastal Regions of Myanmar.

A classification system of the genera *Cladophora*

Phylum : Chlorophyta

Class : Chlorophyceae

Order : Cladophorales

Family : Cladophoraceae

Genus : *Cladophora* Kützing

Species : (i) *Cladophora flexuosa* (O.F. Muller) Kützing

(ii) *Cladophora prolifera* (Roth) Kützing

***Cladophora flexuosa* (O.F. Muller) Kützing**

(Figures. 1 & 2)

Description: Plants are light-green, 0.5-5 cm tall, erect or flexuose, somewhat rigid but delicate primary, rhizoids descending from frond-bases, sometimes septate, 30 μm in diameter, composed of long segments, slightly constricted between each segment; main filaments straight or flexuose, dichotomous or sometimes trichotomous, forming acute axils, 100 - 120 μm in diameter, composed of short segments, 3 - 4 times long provided with thin lateral membrane, 10 - 20 μm thick; branches straight or curved, dichotomous or sometimes opposite, arising from acute axils, 80 - 100 μm in diameter, composed of short segments, 2 - 3 times as long as diameter; branchlets alternate or unilateral, straight or curved, forming acute axils, 60 - 140 μm in diameter, composed of segments 2 - 4 times as long as diameter, with very long top portions, about 5 - 12 segment, ending in obtuse apices; Ultimate branchlets secund or pectinate, arising from every segment or from 2-3 segments, forming somewhat acute axils, straight, long, 20 - 50 μm in diameter, 1-2 segmented, 2 - 4 times longer.

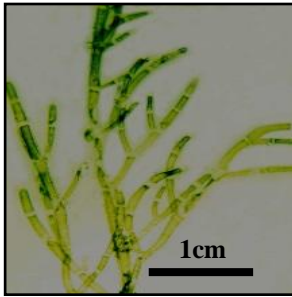


Figure 1. Habit of *C. flexuosa* (O.F.Muller) Kutzing

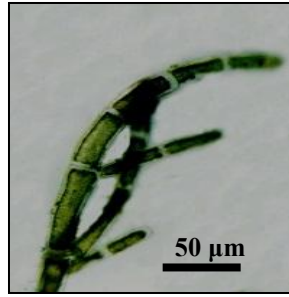


Figure 2. Filament of *C. flexuosa* (O.F.Muller) Kutzing

Spores formation and early germination of the *Cladophora flexuosa* (O. F. Muller) Kutzing (Figs. 3. A-I)

The cell protoplasm of mature thallus changed from yellowish-green to green and later brownish to white due to the abundant spores development (Fig. 3.A). After ten days, the sporangia arise by simultaneous division of the cell. Normally sporangia occurred at the terminal and subterminal of the branch (Fig. 3.B). Each sporangium measured 30-40 μm long and 20-25 μm wide and characterized in brownish colour and spherical and oval shape. After 15 days, cluster of spores were pumped out from the sporangium through a pore. Then individual spores swim from the cluster (Fig. 3.C). Liberated spores were pear shaped and measured 2-3 μm in length. They also swim rapidly with the help of four flagella measuring about 3-4 μm long. The spores settle and round shaped after about 1 minute of free swimming. Some spores and sporangia were embedded in the cell or on the cell wall (Fig. 3.D,E). Germination of released spores was not found until 30 days of watching. On the other hand, embedded spores and spores on the thallus margin gave germination. These embedded spores germinated a new plants. After 20 days, the first germling was measured about 2 μm long (Fig. 3.F). After 25 days, the germling was continuously elongated about 3 μm long. After 30 days, the germling increased to 5 μm long (Fig. 3.G). Some germling were detached from the dying mother plant and grow independently (Fig. 3.H). After 35 days

by cytogenesis two portions were formed, one basal achlorophyllic and the other apical chlorophyllic with a rhizoid (Fig. 3.I). Its cells developed into a mature plant, that is, the chloroplasts became parietal and reticulate and the cell wall became stratified. After 40-50 days, the germling produced the first ramifications.

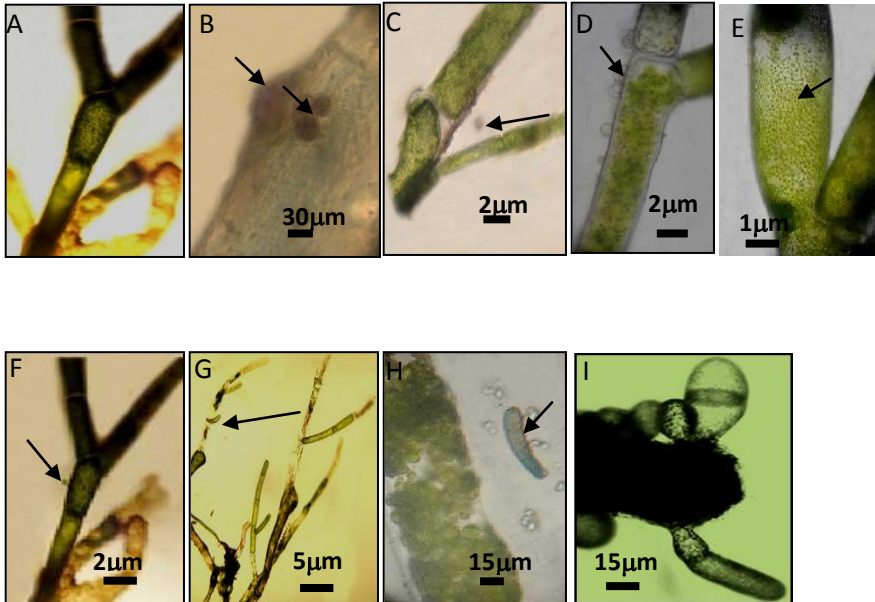


Figure 3. A-I. Spores formation and early germination of *Cladophora flexuosa* (O. F. Muller) Kützing

A. Habit of mature plant; **B.** Formation of sporangia; **C.** Released of spores; **D.** Attached spores on the cell wall; **E.** Embedded spores in the cell wall; **F.** Produced the first germling; **G.** Attached the germling with mother plant; **H.** Detached the germling from mother plant; **I.** Formation of two portions germling.

Local Distribution of *Cladophora flexuosa* (O.F. Muller) Kutzing
(Figure. 4. & Table 1)

Local distribution: Tanintharyi Coastal Region- Hmyawyt, Kampani.

Ayeyarwady and Gulf of Martaban Coastal Region- Kayin Thaug, Setse, Yathae Thaug.

Rakhine Coastal Region- Wetthey Gyaing, Magyi Island.

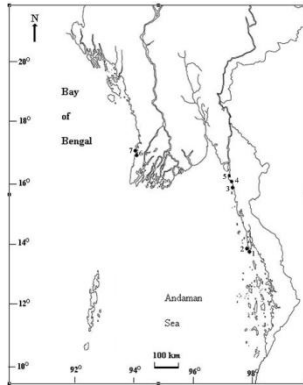


Figure 4. Map showing the collection side of *Cladophora flexuosa* (O. F. Muller) Kutzing along the Coastal Regions of Myanmar. 1. Kampani, 2. Hmyawyt, 3. Yathae Thaug, 4. Setse, 5. Kayin Thaug, 6. Magyi Island and 7. Wetthey Gyaing

Table 1. The distributional range of *Cladophora flexuosa* (O.F. Muller) Kutzing along the Coastal Regions of Myanmar.

Species	TCR		ACR		RCR	
	From	To	From	To	From	To
<i>C. flexuosa</i> (O.F. Muller) Kutzing	Kampani	Hmyawyt	Yathae Thaug	Kayin Thaug	Magyi Island	Wetthey Gyaing
	Lat. 14° 02' N	Lat. 14° 04' N	Lat. 15° 52' N	Lat. 16° 32' N	Lat. 17° 04' N	Lat. 17° 10' N
	Long 98° 04' E	Long. 98° 04' E	Long. 97° 35' E	Long. 97° 36' E	Long. 94° 27' E	Long. 94° 26' E

Abbreviations:

TCR = The Tanintharyi Coastal Region

ACR = The Ayeyarwady and The Gulf of Martaban Coastal Region

RCR = The Rakhine Coastal Region

***Cladophora prolifera* (Roth) Kutzing**

(Figures. 5 & 6)

Description: Thallus is dark green (blackish when dried) in colour coarse, 1-3 cm high, growing as stiff tufts, composed of densely branched fastigate filaments. Old cells in the basal and middle parts of the thallus, each giving off one rhizoid with annular constrictions at their basal poles; these rhizoids grow downwards along the cell or cells below, where they entangle and form a conspicuous stipe that attaches to the substratum. Apical cell division is growing into subsequent cell enlargement. Branching originally acropetally organized, becoming irregular in older parts of the thallus because of intercalary growth. Each subapical cell forms a lateral, often immediately after being cut off from the apical cell; lower down a cell may form as a 2nd or sometimes a 3rd lateral. Apical cells cylindrical with rounded tips, 70-120 μm in diameter 2.5 - 4 times as long as breadth ; cells of the terminal branch systems cylindrical, 130 - 170 μm in diameter, 2- 6 times as long as breadth, increasing towards the base of thallus. Cells of the main axes and basal cells elongated and club-shaped, up to 170 μm in diameter, 5 - 7 times as long as breadth, basal parts often with annular constrictions. Rhizoids are 30 - 70 μm in diameter.

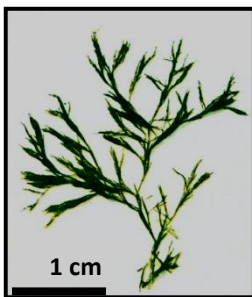


Figure 5. Habit of
C. prolifera
(Roth) Kutzing

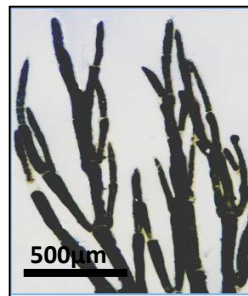


Figure 6. Filament of *C. prolifera* (Roth) Kutzing

Spores formation and early germination of the *Cladophora prolifera* (Roth) Kutzing (Fig. 7. A-I)

The cell protoplasm of mature thallus changed from green to dark-green and later became black due to the spores development (Fig. 7.A). After 5 days, the sporangia arise by simultaneous division of the cell. Normally sporangia occurred at the terminal and subterminal of the branch (Fig. 7.B). Each sporangium measured 50-75 μm long and 20-25 μm wide and characterized in brownish colour and spherical and oval shape. The spores were submerged in sporangia for about 5 days and then released through the pore of the lateral cell wall (Fig. 7.C). Liberated spores from sporangium were pear shaped and measured 1.5-2 μm in length. They also swim rapidly with help of four flagella measuring about 1-1.5 μm long. The spores settle and round shaped after about 2 minutes of free swimming. Some spores and sporangia were embedded in the cell or on the cell wall (Fig. 7.D,E). Germination of released spores was not found until 30 days of watching. On the other hand, embedded spores and spores on the thallus margin gave germination. After 20 days, the first germling was about 2 μm long (Fig. 7.F).

After 25 days, the germling was elongated about 4 μm long. After 30 days and 35 days, the germling was reached to 7 μm and 10 μm long (Fig. 7.G). After 40 days and 45 days the germling was increased up to 20 μm and 30 μm long (Fig. 7.H). After 60 days, the germlings were formed ramification (Fig. 7.I) and after 90 days, it was developed into a mature plant.

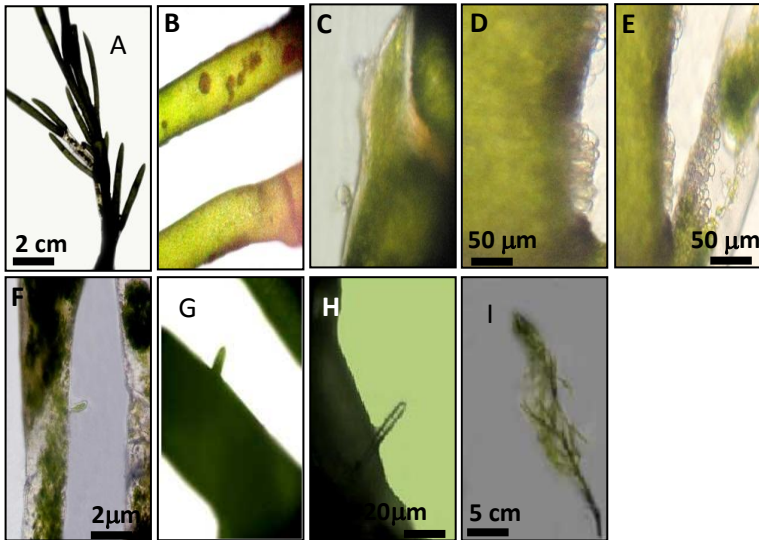


Figure 7. A-I. Spores formation and early germination of *Cladophora prolifera*. (Roth) Kutzing

A .Habit of mature plant; B. Formation of sporangia; C. Released of spores; D. Attached spores on the cell wall; E. Embedded spores in the cell wall; F. Produced the first germling; G. and H. Attached the germling from mother plant, I. Growing of the new plant.

Local Distribution of *Cladophora prolifera* (Roth) Kutzing

(Fig. 8. & Table 2)

Local distribution: Tanintharyi Coastal Region- Kampani, Hmyawyt.

Ayeyarwady Delta and Gulf of Martaban Coastal Region- No data.

Rakhine Coastal Region- Maw Shwe Gyaing, Shwe Ya Gyaing, Wetthey Gyaing, Zee Gyaing and Mawtin Point.

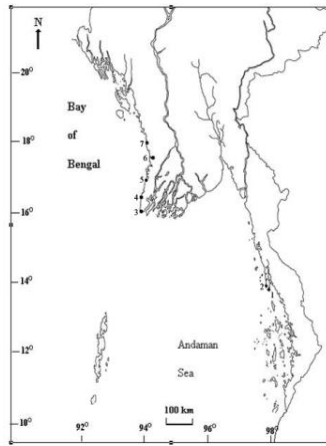


Figure 8. Map showing the collection side of *Cladophora prolifera* (Roth) Kutzing, along the Coastal Regions of Myanmar. 1. Kampani, 2. Hmyawiyit, 3. Mawtin Point, 4. Zee Gyaing, 5. Wetthey Gyaing, 6. Shwe Ya Gyaing and 7. Maw Shwe Gyaing.

Table 2. The distributional range of *Cladophora prolifera* (Roth) Kutzing along the

Species	TCR		ACR		RCR	
	From	To	From	To	From	To
<i>C. prolifera</i> (Roth) Kutzing	Kampani	Hmyawiyit	No data	No data	Mawtin Point	Maw Shwe Gyaing
	Lat. 14° 02' N	Lat. 14° 04' N			Lat. 16° 04' N,	Lat. 17° 48'
	Long. 98° 04' E	Long. 98° 04' E			Long. 94° 20' E	Long. 94° 29'E

Coastal Regions of Myanmar.

Abbreviations:

- TCR = The Tanintharyi Coastal Region
 ACR = The Ayeyarwady and The Gulf of Martaban Coastal Region
 RCR = The Rakhine Coastal Region

Discussions and Conclusion

The genus *Cladophora* was erected by Kutzing (1845, 1853), who described 85 marine species and varieties from along the German and Austrian sea-coasts. Being either unaware of, or disregarding morphological ranges related to age or environment, Kutzing began the taxonomic chaos by describing numerous species and varieties for every new variant he encountered. Unfortunately, most phycologists after Kutzing continued to apply his lax criteria for describing new *Cladophora* species with predictable results.

Van den Hoek (1963) suggested that combined morphological, like history and cytogenesis studies are necessary to resolve the innumerable taxonomical problems in species of *Cladophora*. Most taxonomists in past years have followed Kutzing (1843) and based their taxonomy principals on structural and morphological features (Collins, 1909; Van den Hoek, 1963; Soderstrom, 1963; Van den Hoek and Womersley, 1984). Cytogenesis information and life histories have been largely neglected since and recognized by Valet (1960), such aspects of these species often difficult to investigate. Only integrated studies can provide reliable results, since the morphology of these species are extremely influenced by the environment (Van den Hoek, 1963; Parodi and Caceres, 1991 and 1995).

The species identification of family Cladophoraceae was carried out base on the shape and size of filament, colour in filaments, composed of cell and branching type, shape and size of cell and shape and size of rhizoid. In the present study, two species of *C. flexuosa* (O.F. Muller) Kutzing and *C. prolifera* (Roth) Kutzing have been identified. The largest size of *C. prolifera* (Roth) Kutzing measured about 1.0 - 3.0 cm in height and the largest size of *C. flexuosa* (O.F. Muller) Kutzing was 0.5 - 5.0 cm in height. Moreover, habit of *C. prolifera* (Roth) Kutzing was stiff and tufts, densely branch. The characters of *C. flexuosa* (O.F. Muller) Kutzing was erect or flexuose and di (or) trichotomous branch possessed. The colour of *C. flexuosa* (O.F. Muller) Kutzing was found light-green and olive-green colour. The wide

cells of *C. prolifera* (Roth) Kutzing had (150-200 μm in wide) and the wide cells of *C. flexuosa* (O.F. Muller) Kutzing had (60-100 μm in wide).

In the present study, spores of *Cladophora prolifera* (Roth) Kutzing had ovoid-shaped, equal and quadriflagellate. Spores of *Cladophora flexuosa* (O.F. Muller) Kutzing had pear-shaped, equal and quadriflagellate. The ranges of sporangia were 50-75 μm long, 20-25 μm wide; that of *C. prolifera* (Roth) Kutzing. Sporangia of *C. flexuosa* (O.F. Muller) Kutzing had 30-40 μm long, 20-25 μm wide. Wik-Sjostedt (1970) described *Cladophora flexuosa* (O.F. Muller) Kutzing has a diploid sporophyte with quadriflagellate zoospores and a haploid gametophyte with biflagellate gametes. *Cladophora flexuosa* (O.F. Muller) Kutzing was very easily cultured and every time that spores from newly collected material were put in culture media, they developed into plants. The ranges of spores were 1.5-2 μm of *C. prolifera* (Roth) Kutzing and 2-3 μm of *C. flexuosa* (O.F. Muller) Kutzing. In *C. prolifera* (Roth) Kutzing spores were found abundant in room culture. In *C. flexuosa* (O.F. Muller) Kutzing, spores were found abundant in room culture but not found abundant in incubated culture.

The present study emphasized on the systematic of *Cladophora flexuosa* (O.F Muller) Kutzing and *C. prolifera* (Roth) Kutzing of the genus *Cladophora* Kutzing belonging to the family Cladopharaceae and the order Cladophorales. Seed plants were collected from the three Coastal Regions of Myanmar. In this study, the morphology, spore germination and local distribution of the two species were fully knowledged. *Cladophora flexuosa* (O.F Muller) Kutzing was distribution in Tanintharyi Coastal Region, Ayeyarwady and Gulf of Martaban Coastal Region and Rakhine Coastal Region of Myanmar. *Cladophora prolifera* (Roth) Kutzing was distribution in Tanintharyi Coastal Region and Rakhine Coastal Region of Myanmar. *Cladophora flexuosa* (O.F Muller) Kutzing mainly predominate along the Ayeyarwady and Gulf of Martaban Coastal Region and *Cladophora prolifera* (Roth) Kutzing mainly predominate along the Rakhine Coastal Region of Myanmar.

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