Study on *Rhizoclonium riparium* (Roth) Harvey from three coastal regions of Myanmar

Moe Moe Khaing*

Abstract

Genus *Rhizoclonium* belonging to the family Cladophoraceae, Chlorophyta.It was from collected from three coastal regions of Myanmar from 2014 to 2016. This algae was identified as *Rhizoclonium riparium* (Roth) Harvey by Murray, 1887; Silva et.al., 1996; Leliaert, 2004; Dawson, 1954; Durairatnam, 1961. The descriptions of taxon with emphasis on the shape, size, colour and type of cell and rhizoid. Culture studied on spores formation and germination of *Rhizoclonium riparium* (Roth) Harvey. In addition, the distribution ranges of *Rhizoclonium riparium* (Roth) Harvey along the three coastal regions of Myanmar was presented.

Keywords: Morphology, Laboratory culture, Local distribution.

Introduction

The algae variously ranked as an order, a class, a division or a group in different systems of classification, is an assemblage of chlorophyll-bearing autotrophic. Thallophytes, bounded by a cell wall, made up of pure or mixed carbohydrates. Algae of the seawater are called marine algae. They grow generally in waters in which solutes are usually 30 - 44 %. Marine vegetation occupies only about 2 % of the general surface (shorelines and zone of relatively shallow water) of the sea, of which a large part is occupied by the phytoplankton, mainly of the diatoms and dinoflagellates. Important contributions had been made on the life-cycles of algae by many phycologists (Sharma, 1998).

The Cladophoraceae are found both in freshwater and in the sea, where they inhabit mainly the rocks of the littoral zone; some, like the tropical, *Rhizoclonium riparium* Harvey may occur on terrestrial substate beyond reach of the sea, while it is commonly found on sand-covered rocks or even on pure sand, acting as a binder, as well as on salt-marshes. This preference for sandy pools is also shown by other members of the family. The threads of *Rhizoclonium* are often altogether unbranched, though in other cases (commonly in the *R. riparium*) there are a number of very short, almost colourless, one-or few-celled branchlets of irregular shape which resemble, but do not function as rhizoids and are responsible for the generic name (Fritsch, 1961).

There are 69 species names in the genus *Rhizoclonium* at present, of which 25 have been flagged as currently accepted taxonomically (Guiry, 2013). The characters of genus *Rhizoclonium* are filaments slender, 60 μ m in diameter, loose lying with basal cells, or attached by holdfast with basal lobes. Thallus of the plant unbranched and with one-to few-celled rhizoidal laterals. The length of the cells is several to many times longer than broad with numerous nuclei. Chloroplast reticulate, parietal, with pyrenoids, often densely packed with starch. Reproduction in freshwater species is by fragmentation and rarely biflagellate zoospores. In marine/brackish species, isomorphic life history with quadriflagellate zoospores and biflagellate gametes had been reported from 2 species.

^{*} Department of Botany, Maubin University.

In Myanmar, Kyaw Soe and Kyi Win (1977) reported the occurrence of two species of *Rhizoclonium* along the Coastal Region of Myanmar. Moreover, Soe-Htun et. al. (2009) accounted, the two species of *Rhizoclonium*, viz., *R. africanum* Kutzing and *R. grande* Borgesen along the three Coastal Regions of Myanmar.

The purposes of this study are: 1) to identify the *Rhizoclonium riparium* (Roth) Harvey, 2) to study the reproductive structures, 3) to record the distributional ranges of *R. riparium* (Roth) Harvey along the three coastal regions of Myanmar.

Materials and methods

The samples were collected from littoral zone, brackish water and intertidal zone, along the three Coastal Regions of Myanmar from 2014 to 2016. Fresh and living plants were collected from several localities and saved in ice box and brought to the laboratory of Mawlamyine University for observation. Some specimens were fixed and preserved in 5 % formaldehyde solution which was prepared with seawater. 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

Rhizoclonium riparium (Roth) Harvey was collected from the three Coastal Regions of Myanmar.

A classification system of the genera Rhizoclonium

Phylum : Chlorophyta

Class : Chlorophyceae

- Order : Cladophorales
- Family : Cladophoraceae
- Genus : Rhizoclonium Kutzing

Species : *Rhizoclonium riparium* (Roth) Harvey.

Morphology of Rhizoclonium riparium (Roth) Harvey.

Plants uniseriate, unbranched; yellowish-green to green, on drying to olive-green, the filaments curled; entangled thin mats or strands, up to 30 cm long, sometimes twisted with numerous short filaments, tapering, irregular rhizoidal branches, composed of 2-5 cells. The cells are cylindrical, 10-20 µm in diameter,

3-5 times long as diameter and cell walls 2 μ m thick; chloroplast coarsely reticulate with numerous pyrenoids; Zoosporangia observed in which all were slightly enlarged at their upper ends and provided with a discharge pore. Thallus is small, floating mats, entangled with other algae (particularly *Enteromorpha*), mud dwelling or epiphytic plants "coating on mangroves" (Fig. 1 & 2).

Main characteristics of this species are observed as follows:

- (1) The plants are soft, composed of cylindrical cells;
- (2) The filaments straight to irregularly curve;
- (3) The filaments regular with one-five celled;
- (4) Tapering rhizoidal branches.



Figure 1 Habit of *R. riparium* (Roth) Harvey



Figure 2 Filament of *R. riparium* (Roth) Harvey

Spores formation and germination of the *Rhizoclonium riparium* (Roth) Harvey

Mature plants were used as seed plants. These plants were inoculated in each petridish (6×2 cm) containing 20 ml of 15 ‰ enriched seawater. Culture petridishes were incubated under 16 Light: 8 Dark photoperiod in incubators and in room temperature for 60 days. Early development of sporelings was examined and the diameter (in length) of sporangia was measured under electron microscope with the help of ocular meter at 5 days intervals. Culture medium was changed after each examination.

Mature filaments were gradually changed in colour from yellowishgreen to green and further to dark-green (Fig. 3). The sproangia arised by simultaneous division of the cell contents into numerous parts. Normally sporangia formation was intercalary cells of the filament (Fig. 4). The fertile portions changed in colour, green to black during the formation of reproductive organs. From the end of the cell divisions as previously mentioned above in a matured vegetative cell. Each sporangium measured 240 - 250 μ m long and 10 μ m - 15 μ m wide and characterized in slender shape, is always detached from the thallus. The cell walls became hyaline due to the abundant spores development. Many spores were submerged in the sporangia for about 7 days and then released through a single median pore in the cell wall and then, individual spores swim from the cluster to anywhere (Fig. 5, 6). Some spores were embedded in the cell and on the cell wall. Liberated spores from mother plants are ovoid shaped and measured 1.5 - 2 μ m in length. They also swim rapidly with the help of four flagella measured about 2 - 3 μ m long. About 1 min of free swimming after being released, they settled and rounded in shape. 30 days after, the germination was not found from the released spores. On the other hand, embedded spores and spores of the filament margin gave germination. About 17 days later, the first germling was elongated about 4 μ m long (Fig. 7). After 22 days and 27 days, the germlings increased to 15 μ m and 25 μ m long (Fig. 8). After 32 days and 37 days, the germlings reached to 50 μ m and 100 μ m long (Fig. 9). By cytogenesis, two portions were formed, one basal with achlorophyllic and other apical with chlorophyllic, after 45 days. The latter began to be an elongated cell which reached to 200 μ m after 55 days. Then, it began to divide elongatedly and its cells formed a mature filament. The chloroplasts became parietal and reticulate in the cell walls.



Figure 3 Habit of mature filament

Figure 4 Formation of sporangia

Figure 5 Released of spores

um

Figure 6 Formation of many spores in nearly on the cell wall

25 µm

100 um

Figure 7 Produced the first germling, after 17 days

Figure 8 Elongation of the germling, after 27 days

Figure 9 Elongation of the germling, after 37 days

Distribution of Rhizoclonium riparium (Roth) Harvey

Local distribution of genus *Rhizoclonium riparium* (Roth) Harvey was studied in three coastal regions (Fig. 10).

Tanintharyi coastal region - Kampani, Hmyawyit, Thabawseik.

Ayeyarwady delta and Gulf of Mottama (Martaban) coastal region – Yathae thaung, Setse, Kayin thaung, Kyaikhami, Kadonepaw.

Rakhine coastal region - Magyi, Wetthey gyaing, Maw shwe gyaing.



Figure 10 Map showing the collection sides of *Rhizoclonium riparium* (Roth) Harvey along the three Coastal Regions of Myanmar. 1. Kampani, 2. Hmyawyit, 3. Thabawseik, 4. Yathae thaung, 5. Setse, 6. Kayin thaung, 7. Kyaikkhami, 8. Kadonepaw, 9. Magyi, 10. Wetthey gyaing and 11. Maw shwe gyaing.

Discussions and conclusions

Rhizoclonium riparium(Roth) Harvey plants growing in abundant layers spreading considerably on stone and rocks. Myanmar, North America, Ceylon and Karachi species are yellowish-green in colour (Anand, 1940; Abbott and Hollenberg, 1976; Borgesen, 1936; Taylor, 1967). Hawaiian islands specimens are pale-green in colour (Abbott & Huisman, 2004) and dark green colour in California species (Abbott & Hollenberg, 1976). Myanmar species are associated with *U. compressa*. The length of the specimens from Myanmar and Hawaiian islands are up to 30 cm. Sometimes Myanmar species are observed as a coating on mangroves roots. Karachi specimen cells are 12 - 14 μ m in diameter with 3-5 times as long as breadth and devoid of rhizoid. California specimens are 23 - 27 μ m in diameter with 1 - 2.5 times long as breadth. Rhizoids are branched and tapering. Hawaiian Islands materials are 10 - 70 μ m in diameter, breadth 1 - 6 times longer and

rhizoids never rebranch. Ceylon plant cells are 20-30 µm in diameter, with 1-5 times as long as breadth. Tapering rhizoids, branches are 20-30 µm in diameter. In Myanmar species the cells are 10-20 µm in diameter, 3-5 times long, rhizoids, tapering, irregularly branched, composed of 2-5 cells. The Myanmar species are more slender and longer (Abbott & Hollen berg, 1976; Abbott & Huisman, 2014; Silva et.al., 1996; Leliaert, 2004; Anand, 1940; Borgesen, 1936; Durairatnam, 1961; Magruder, 1979; Dawson, 1954; Murray, 1887; Taylar, 1967).

Rhizoclonium riparium(Roth) Harvey which is found in Kyaikkhami, Setse and Kadone-paw villages are in the Ayeyarwady and Gulf of Martaban Coastal Region. Among these three areas, the Kyaikkhami specimens were abundant and exceptionally long compared with other areas during the rainy season. They were also found coated on the mangrove roots and along old ruined boats.

Rhizoclonium riparium(Roth) Harvey was ubiquitous in Kamake, Tawkapo and Saibalar areas and Setse and Kyaikkhami areas of Ayeyarwady delta and Gulf of Mottama coastal region (Soe-Htun et.al., 1999). *Rhizoclonium* spp. was dominated abundantly in the mangroves swamps of Setse area at the Mouth of the Gulf of Martaban (Mottama) (Soe-Htun, 1998).

In the four months duration of this research vegetative and asexual reproduction were distinct. *Rhizoclonium riparium* (Roth) Harvey took a period of 10 days from young filament to mature filament. Spores were produced due to the distinct coloration and morphology. The measurement at the first germling was 4μ m long. At the period of 37 days the germling reached to 100 μ m long. By cytogenesis, two portions were formed after 45 days. At first, the growth rate in length was slow but after 20 days the growth rate became rapid. Spores were found abundant in room culture and limited in incubated culture. The growth rate of the new plants was slow in room culture than in incubated culture which was constant.

Among the *Rhizoclonium riparium*(Roth) Harvey filaments, the largest size of filament measured up 30 cm long, straight to irregularly curved and yellowish-green to green in colour. Prominent characters were plants soft, freely solitary with cells less than 20 μ m in diameter *Rhizoclonium riparium*(Roth) Harvey. The main character of *R. riparium* (Roth) Harvey has a irregular branch rhizoids.

In this study, distribution ranges of *Rhizoclonium riparium* (Roth) Harvey was found in Rakhine coastal region from Magyi to Maw Shwe Gyaing, Ayeyarwady delta and Gulf of Mottama coastal region from Yathae Thaung to Kadonepaw, Tanintharyi coastal region from Kampani to Thabawseik (Table 1).

Sr. No	Name of coastal regions	Latitude and Longitude		Name of sites	
		From	То	From	То
1	Tanintharyi Coastal Region	Lat.14°02'N and Long.98°24'E	Lat.14°05'N and Long.98°25'E	Kampani	Thabawseik
2	Ayeyarwady and Gulf of Mottama Coastal Region	Lat.15°52'N and Long.97°35'E	Lat.16°12'N and Long.97°38'E	Yathae Thaung	Kadonepaw
3	Rakhine Coastal Region	Lat.17°04'N and Long.94°27'E	Lat.17°48'N and Long.94°29'E	Magyi	Maw Shwe Gyaing

Table 1 Distribution range of *Rhizoclonium riparium* (Roth) Harvey along the three coastal regions of Myanmar.

In conclusion, morphological features were changed in response to various environmental conditions. Temperature and salinity played an important role in the growth and reproduction of Cladophoraceae. These factors had determined the large-scale boundaries of algal distribution patterns and in the development of ecotypes.

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