Structure and Reproduction of *Grateloupia filicina* (Halymeniaceae, Rhodophyta) from Indian Coast

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The vegetative and reproductive features of *Grateloupia filicina* (Lamouroux) C. Agardh (Cryptonemiales, Halymeniaceae) from different parts of the Indian Coast were studied. The plants grow in a wide range of habitats and showed a lot of morphological variations. The development of the thallus is multiaxial type and the medullary region is composed of irregular, branched and stellate shaped cells. The gametophytic plants are dioecious and the male plants are smaller compared to female plants. The carpogonial branch is two-celled and formed on an accessory branch system known as ampulla. Cystocarps are spherical to subspherical with distinct ostioles and scattered on the thallus surface. Tetrasporangia are common and tetraspores are either cruciate or decussate. Bisporangia are occasionally encountered. Our study suggests occurrence of two intraspecific taxa of *G. filicina* i.e. var. *luxurians* and var. *filicina* from Indian coast.

**Key Words:** development, *Grateloupia filicina*, reproduction, structure

INTRODUCTION

The red algal genus *Grateloupia* C. Agardh is the largest genus of the family Halymeniaceae (Cryptonemiales). The plants are widely distributed all over the world but are abundant in tropical and subtropical regions. Nearly 40 species of *Grateloupia* have been reported from different parts of the world (Kylin 1956; Kraft 1977). *Grateloupia* presently includes the widest assortment of plant habits of any of the genera, ranging from the finely pinnate *G. filicina* (Taylor 1960), through sub-dichotomous forms like *G. hawaiiana* (Dawson 1958), to foliose blades such as *G. comorinii* (Boergesen 1938) to large polymorphic species like *G. doryphora* (Villalard-Bohsack and Harlin 1997 and 2001). Out of all the species, *G. filicina* is more important as the plants are cultivated as a source of food and carrageenan in many countries (Tokuda et al. 1987; Zablackis 1987; Bula-Meyer 1989; Chen and Chiang 1994; Sahoo 2000). This species has a carrageenan similar to lambda type but is distinct in its physical properties and proportions of sulfate and 3, 6- anhydrogalactose (Zablackis and Perez 1990).

The generitype, *G. filicina* was originally published as *Delesseria filicina* (Lamouroux 1813) and later transferred to *Grateloupia* when the genus was established by Agardh in the year 1822. Kylin (1930) published a full and detailed account of the developmental morphology of *G. filicina*. Chiang (1993) studied the developmental sequences of *G. filicina* in culture from Taiwan. Chen and Chiang (1994) studied the protoplast development in *G. sparsa* and *G. filicina* from Taiwan. Recently Kawaguchi et al. (2001) did a comparative study on *G. filicina* from North west pacific with *G. asiatica* from Mediterranean. Although a number of studies have been done on different aspects of *G. filicina*, the species remains still very controversial as it has nearly nine intraspecific taxa. Further there is no detailed study on this species from the Indian region although six species of *Grateloupia* have been reported from different parts of the Indian coast (Sahoo et al. 2001). So in the present study we have undertaken a detailed study on the vegetative and reproductive structures of *G. filicina* from different parts of the Indian coast. Our study suggests that there are at least two intraspecific taxa of *G. filicina* growing in Indian coast.

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MATERIALS AND METHODS

The plants of *Grateloupia filicina* (Lamouroux) C. Agardh were collected from different parts of the Indian Coast (Fig. 1) such as Mandapam, Tamilnadu (09°06'–09°14'N and 78°53'–79°24'E); Kollam, Kerala (08°53'–08°57'N and 76°32'–76°34'E); Baga (Goa) and Chilika lake, Orissa (19°28'–19°54'N and 85°6'–85°35'E). The voucher herbarium specimens were prepared and submitted in the Herbarium at Department of Botany, University of Delhi, Delhi. The thalli were fixed in the field in 4% formalin/seawater as well as 10% Acrolein. For the light microscopic studies selected parts of the Acrolein fixed materials were transferred successively through 2-methoxyethanol (2 times for 24 hrs each); 100% ethanol (for 24 hrs); n-propanol (24 hrs) and n-butanol (24 hrs) at 4°C. Infiltration was done for 7 days in plastic monomer mixture (Feder and O'Brien 1968). The monomer mixture was changed after every 24 hrs. After infiltration, embedding was done in gelatin capsules filled with monomer mixture and polymerization was done at 40°C for 24 hrs and at 60°C for 48 hrs in a temperature controlled oven. Sectioning was done on A.O. spensor 820 rotary microtome and 2 μm thick sections were cut. The sections were stained with Toluidine Blue O for carboxylated and sulphated polysaccharides (McCully 1966); periodic acid for insoluble polysaccharides (Feder and O'Brien 1968) and Coomassie Brilliant Blue for proteins (Weber and Osborn 1975). Pictures were taken in a Nikon E-600 Photomicroscope.

For the Scanning Electron Microscopic studies selected parts of the specimen were fixed in 3% glutaraldehyde prepared in 0.025 M phosphate buffer at PH 6.8 for 8 hrs. Subsequently materials were passed in graded cold ascending acetone series ranging form 10-100% at room temperature. After dehydration, critical point drying was done and samples were mounted on aluminium stubs and left overnight in a desiccator containing silica gel. Subsequently the material was coated with pure silver under vacuum with a thickness of 350 A° and then observed under a Philips 501-B Scanning Electron Microscope under high vacuum.

RESULTS

Habitat: In Mandapam, *Grateloupia filicina* are found growing on small rocks, plastic bags, ropes etc. in polluted area where the village sewage gets discharged whereas the plants from Goa and Kollam grow on granitic and laterite rocks in clean non polluted sea. The seawater temperature ranges between 24-32°C whereas the salinity was between 30-33 ppt. But in Chilika Lake the plants grow on small rocks in muddy brackish water where the water temperature ranges between 24-29°C and the salinity was between 3-15 ppt.

Morphology: Plants are bushy deep reddish brown to greenish in colour. Thalli are composed of several upright blades, developing from a common discoid holdfast (Figs 2B-D). The main axis is round at the bottom and flattened towards the apex which branches extensively (Figs 2B-D). Branches are long, linear, tapering towards the apex as found in the Mandapam material (Fig. 2A) or bifurcate only once as found in the material collected from Chilika lake (Fig. 2B). Sometimes the axis are beset with numerous proliferations that are irregularly arranged along the margins. These proliferations 2-4 cm long and sometimes produce second order of small proliferations. The plants collected from four different places during the present study showed differences in their morphology. The plants collected from Chilika Lake, Orissa are flat, thin, bushy and dichotomously branched and grow up to a height of 10.5 cm (Fig. 2B). The plants collected from the other three locations are less bushy and grow up to 12 cm in height. Epiphytes like *Ulva lactuca*, *Ceramium elegans*, *Coconoides*
scutellum and Nostoc sp. were found growing luxuriantly on the thallus.

Vegetative thallus: The development of the thallus is multiaxial or fountain type. Internally the thallus is differentiated into a single layered epidermis, a compact cortex and loose filamentous medulla (Fig. 3A). A thick extracellular layer covers the epidermis which stains deep purplish blue with Toludine Blue O (TBO) and deep magenta with Periodic Acid Schiff (PAS) reagents. These staining reactions suggest that the layer contains a high amount of insoluble polysaccharides thus is mucilagenous in nature. The epidermal cells are elongated, thin walled, active and cut off the cells towards the cortical side. The cortex is 5-6 layered and is divided into
Fig. 3. A-F. Vegetative and reproductive structures
A. Transverse section of the thallus showing epidermis, cortex and medulla. scale bar = 100 μm; B. Magnified view of medullary region showing irregular shaped hyphal cells. scale bar = 50 μm; C. Scanning electron micrograph showing the medullary region; D. Transverse section of apical part of proliferations showing development of spermatangium from outer cortical cells. scale bar = 20 μm; E, F. Magnified views showing the development of spermatangia and release of spermatia. scale bars = 20 μm, 10 μm (c - cortex, e - epidermis, m - medulla, pc - pit connections, sp - spermatium, spm - spermatangial mother cell)
inner and outer cortex. The outer cortex is compact, 2-3 layered and composed of small rounded, isodiametric cells towards the epidermis (Fig. 3A); whereas, the inner cortical region is loose, 2-3 layered and composed of fairly large spherical and vacuolated cells, which increases in size towards the center. The cells are connected to each other by pit connections (Figs 3F, 5A), which stain distinctly with Coomassie Brilliant Blue indicating that it is proteinaceous in nature.

Medulla is proportionately large and somewhat loose in texture (Figs 3A-C). The inner medullary region is composed of loosely interwoven longitudinal filaments. These cells are irregular, branched and stellate shaped with long interconnecting and irregular outlines that are connected with each other (Figs 3B, C). The medullary region is filled up with large amounts of sulphated and carboxylated polysaccharides as they stained positively with TBO (Fig. 3B). Usually the thallus is solid and compact at the apical region whereas it is circular at basal region.

Reproductive structures

The gametophytic plants are dioecious. Male plants are smaller and more sparsely branched and bear fewer proliferations compared to the female plants. Spermatangia are usually scattered. Tetrasporophytic plants are denser than carposporophytic plants. The later can be easily distinguished to the naked eyes as the cystocarps are scattered on the thallus surface as pink spots whereas the tetrasporic plants can be identified only under the microscope which shows a number of cream coloured tetrasporangia (Figs 5C, D). In Mandapam material, the tetrasporic and cystocarpic plants are found between October to February while a peak sporulation was found in the month of January. Tetrasporic and cystocarpic plants could only be seen in September in Kerala and Goa materials. Interestingly no cystocarpic plants were found in Chilika material from January to October however only tetrasporic plants could be collected during June.

Spermatangium: Spermataangia are found in the proliferations and are produced from the outer cortical cells (Figs 3D-F). The outermost cortical cells get elongated and function as spermataangial mother cells. Usually only one spermatangium is seen on each mother cell, but sometimes two are also found (Figs 3D, E). The spermata get released by the dissolution of the gelatinized cell walls through the outer surface (Fig. 3F). The spermata are ovoid spherical in shape and creamish in colour. They are thin walled and have a distinct nucleus, which occupies the upper portion of cell.

Carpogonium: The carpogonium and auxiliary cells are formed in separate accessory branch systems known as ampulla. The ampulla arises as accessory laterals of inner cortical cells. The primary ampulla consists of 6-9 sub-spherical cells which gives rise to secondary ampullary filament (Fig. 4A). The basal cells of the secondary ampulla are sub-spherical, which progressively becomes smaller towards the tip. The carpogonial branch is two celled containing basal hypogonous cell and a carpogonium developing on a primary ampullary filament (Figs 4A, B). The hypogonous cell develops from the third or fourth cell of the primary filament and bears a side branch. The carpogonium is flask shaped (Fig. 4B) and bears a trichogyne. The trichogyne increases in length and when fully developed is thick walled, 8-13 times longer than the carpogonium.

Auxiliary cell: The auxiliary ampulla is of independent origin arising from an inner cortical cell, situated a little away from the carpogonial ampulla (Fig. 4B). The inner cortical cell divides and gives rise to primary and secondary filaments but tertiary filaments are never produced. The primary filaments consists of 6-8 sub-spherical cells, out of which the third or fourth cell acts as auxiliary cell. The auxiliary cell is comparatively large, ovoid to sub-spherical, uninucleate and contains dense protoplasm.

Post Fertilization Development: After fertilization, the trichogyne is first cut off from the rest of the carpogonium. Then the fertilized carpogonium gets enlarged and produces two to many connecting filaments. These connecting filaments which are thick walled and non-septate subsequently connect to the auxiliary cell, thus establishing a direct contact. At the point of contact the membrane gets dissolved and the diploid nucleus from the connecting filaments get transferred to the auxiliary cell. Soon after, the diploid nucleus and the auxiliary cell nucleus divide and one of the diploid nuclei passes into an outgrowth of auxiliary cell which cuts off as gonimoblast initial, whereas the other three nuclei remains inside the auxiliary cell. The gonimoblast initial, which faces towards the surface of the thallus divides and forms a number of gonimoblast cells. The lower cell of the gonimoblast filament near the gonimoblast initial cell remains sterile, where as the upper cell develops into carposporangia. Each carposporangium produces a single carpospore. Concurrent with gonimoblast development, the auxiliary cell produces
Fig. 4. A-F. Carposporangium and cystocarp development
A. A mature carpogonium showing a hypogynous cell, a carpogonium enclosed by primary ampullary filament. scale bar = 20 μm; B. A mature auxiliary cell ampulla. scale bar = 20 μm; C. A mature cystocarp embedded in the thallus showing carpospores, gonimoblast cells and fusion cells. scale bar = 100 μm; D. Scanning electron micrograph of cystocarps showing ostiole and empty cystoricpic cavity; E. Surface section of thallus showing empty cystocarpic cavities and ostioles; F. Mature cystocarp showing compact arrangement of carpospores which are tightly held due to mucilage (ac - auxiliary cell, cp - carpogonium, cr - carpospores, cy - cystocarp, ec - empty cystocarpic cavities, o - ostiole)
lateral branches, which form thick involucral network of filaments around the young gonimoblasts forming a cystocarp (Figs 4C, F). With the growth of carposporophyte, the cystocarps are pushed deeper into the hollow centre of the thallus (Fig. 4E). The cystocarps are spherical to sub-spherical and found scattered over the surface of the thallus (Fig. 4E). Each cystocarp has an ostiole through which the carpospores get released (Figs 4C, D). The carpospores after release leave empty cystocarpic cavities as depressions on the thallus surface (Figs 4D, E).

**Tetrasporangium:** Like carpogonial and auxiliary ampullae, the tetrasporangia arise as secondarily developed one-called lateral branch from the cells of the inner cortex. The tetrasporangium is cut off by a curved wall from the upper portion of the mother cell (Fig. 5A). It soon enlarges and attains an elongate ellipsoidal cell. After reaching a fairly large size, it divides transversely into two. Each of the two daughter cells thus formed, divide vertically to form four spores. Depending on whether the vertical divisions are both in the same plane or perpendicular to each other, the tetraspores are cruciately or decussately arranged (Figs 5B-D). Occasionally, bisporangia are also found which are two-celled structure with a distinct cleavage furrow (Fig. 5C).

**DISCUSSION**

Numerous discrepancies and contradictions exist in the literature with respect to diagnostic characters of genera in the family Halymeniaceae. Kraft (1977) stated that the genus Grateloupia is poorly defined and better criteria are needed to understand the taxa. The species of Grateloupia show a lot of similarities and variations in their thallus structure. Distinct morphological variations are found within a single species when the plants were collected from different geographical locations as well as within a single population. Wide range of morphological variations have been reported in *G. doryphora* within the population collected from same locality in Rhode Island, USA (Villard-Bohnsack and Harlin 1997 and 2001). Similar morphological variations have also been observed in *G. indica* within a population collected from the same geographical location at port Okha, Gujarat, India (Balakrishnan 1961). Whereas in Japan and northern China *G. asiatica* has long been known as *G. filicina* due to their gross morphological similarity (Kawaguchi et al. 2001). Several intraspecific taxa in *G. filicina* have been reported from various parts of the world till today based on their morphology and other characters. Montagne (1836) reported var. *ramentacea* from France; Castagne (1845) reported var. *cylindricaulis* and also var. *simplex* from the Mediterranean; Kützing (1847) reported var. *elongata* from Java; Gepp and Gepp (1906) reported var. *luxurians* from Southern Australia; Howe (1924) reported var. *lomentaria* from Shangdong Province in Northern China; Okamura (1936) reported var. *porraceae* from West Indies; Boergesen (1935) reported var. *cirrhosa* from India and Mazaa (1915) reported var. *hawaiiana* from Hawaii, USA.

In the present study, thalli of *G. filicina* showed a lot of morphological variations and falls into two categories. The plants collected from Chilika Lake seems to be variety *G. filicina* var. *luxurians* with the similar characteristics of *G. filicina* var. *luxurians* reported from Australia (Irvine 1983). Whereas, the plants collected from Goa, Kollam and Mandapam showed a distinct resemblance with each other and are having characters of *G. filicina* var. *filicina*. Such morphological variations may be due to different ecological parameters of which the salinity is an important factor. As mentioned in observation, that the plants in Chilika grows in a brackish water lake where the salinity ranges between 3-15 ppt and have no wave actions compared to the other three locations where salinity is between 30-33 ppt and strong wave actions. However, further investigation is required in this aspect.

In the present study, all the plants collected from Indian Coast are dioecious although their reproduction timings are different. Interestingly so far all the varieties of *G. filicina* reported from different parts of the world are dioecious. Kawaguchi et al. (2001) reported dioecious plants in *G. asiatica* but rarely monoecious plants are also reported. The growth of thallus is multiaxial which has also been reported in other species of *Grateloupia* (Balakrishnan 1961; Kraft 1977; Villard-Bohnsack and Harlin 1997). In *G. filicina* (present study) spermatangia are produced from the outermost cortical cells in proliferations, and are terminal on the mother cells. Only one spermatangium is present on each mother cell, but sometimes two are also found. Balakrishnan (1961) reported occurrence of spermatangia on the outermost cortical cells in *G. lithophila*. Wang et al. (2000) have also reported production of spermatangia from the outermost cortical cells in *G. catenata*.

A two-celled carpogonial branch has been reported in all the materials collected from different parts of the Indian coast. Reports of two-celled carpogonium branch
Fig. 5. A-D. Tetrasporangium development
A. Transverse section of thallus showing tetrasporangial mother cell and bicelled stage of tetrasporangium (arrow). scale bar = 10 μm; B. A mature tetrasporangium. scale bar = 10 μm; C. Scanning Electron micrograph showing a bisporangium and a tetrasporangium; D. Scanning Electron micrograph showing tetrasporangia lying on the surface of the thallus after release (bs-bisporangium, t-tetrasporangium, pc - pit connections, tsm - tetrasporangial mother cell)

has also been found in G. indica, G. comorinii, G. lithophila (Balakrishnan 1961); in G. intestinalis (Kraft 1977); in G. catenata (Wang et al. 2000) and in G. asiatica, G. filicina (Kawaguchi et al. 2001). It seems two-celled carpogonial branch is an universal character not only in Grateloupia but also amongst the members of Grateloupiaeae.

However, Fritsch (1945) reported a 3-celled carpogonial branch in Gloeosiphonia whereas Goff and Coleman (1984) reported a 4-celled carpogonial branch in Choreocolax polysiphoniae (Choreocolaceae).

In cryptonemiales, the carpogonia and auxiliary cells are produced on distinct branch systems and fertilization
is always followed by the transfer of the zygote nucleus to an auxiliary cell, which is situated away from carpogonium (Fritsch 1945). Similar types of developments are reported in *G. filicina* in the present study where the carpogonium gives rise to connecting filaments, which fuse with the auxiliary cell. The auxiliary cell receives the diploid nucleus through the connecting filament. However, in some species of *Grateloupa* the connecting filaments continue to grow beyond their junction with an auxiliary cell (Berthold 1884; Balakrishnan 1949, 1961) whereas in other species, the connecting filament ceases growth at fusion with the auxiliary cell (Balakrishnan 1961; Kawabata 1954; Wang et al. 2000; Kawaguchi et al. 2001). This seems to be a variable feature in the genus. In the present study reproductive details, particularly the shape of the carpogonial and auxiliary cell ampulla are less variable and are consistent with earlier descriptions of the genus and family (Sjostedt 1926; Fritsch 1945; Balakrishnan 1961; Chiang 1970).

In *G. filicina* (present study) tetrasporangial initials are cut off from the third or fourth cortical cells from the surface. Mature tetrasporangia are ellipsoidal and are either cruciate or decussate. Similar observations have also been found in *G. indica*, *G. comorinii*, *G. lithophila* (Balakrishnan 1961); *G. intestinalis* (Kraft 1977); *G. catenata* (Wang et al. 2000); and *G. asiatica* (Kawaguchi et al. 2001). In the present study we reported the occurrence of bisporangia for the first time in *G. filicina* which has not been earlier reported in the genus. Although bisporangia are reported in some members of of Corallinaceae and Ceramiaceae such as *Lithophyllum*, *Lithothamnion*, *Amphiroa*, *Crovania*, *Reinboldiella*, *Callithamnion* and *Seirospora* etc. Itono (1977) conjectures that bisporangia are homologous with tetrasporangia. After the first cleavage the subsequent cleavages are either absent or delayed. There have been no reports about the occurrence of bisporangia in *Grateloupa* earlier. So this point needs further clarification and reinvestigation. Although our studies clearly defined two intraspecific taxa from Indian region a comprehensive study is still required in *G. filicina* from different geographical regions of the world to clarify their true status.

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