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Morphology, anatomy and reproduction of the populations of a new alga Gelidium usmanghanii (Gelidiales, Rhodophyta)

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RÉSUMÉ

AFAQ-HUSAIN, S. & M. SHAMEEL (1996). Morphologie, anatomie et reproduction dans les populations d'une algue nouvelle Gelidium usmanghanii (Gelidiales, Rhodophycées). *Candollea* 51: 433-443. En anglais, résumés français et anglais.

Description d'une nouvelle algue rouge de la côte du Pakistan: *Gelidium usmanghanii* Afaq-Husain & Shameel. Etude critique de ses structures végétative, anatomique et reproductive. Ces plantes se distinguent des autres *Gelidium* par leur aspect caractéristique: enroulement en spirale de leurs frondes aplaties, ondulations marginales et croissance rigoureuse des proliférations marginales diversément ramifiées. Une taille réduite, une texture papyracée et une ramification irrégulière sont également typiques de cette espèce.

ABSTRACT

AFAQ-HUSAIN, S. & M. SHAMEEL (1996). Morphology, anatomy and reproduction of the populations of a new alga Gelidium usmanghanii (Gelidiales, Rhodophyta). *Candollea* 51: 433-443. In English, French and English abstracts.

A new species of red algae, *Gelidium usmanghanii* Afaq-Husain & Shameel has been described from the coast of Pakistan. Its vegetative, anatomical and reproductive structures have been studied critically. These plants may be distinguished from other species of *Gelidium* by their characteristic appearance compounded by spiral coiling of flat fronds, marginal undulation and dense growth of marginal proliferations, which are variously branched. The small size, papery texture and irregular branching are other characteristic features of this species.

KEYWORDS: Marine algae – *RHODOPHYTA* – *GELIDIACEAE* – Karachi – Pakistan – Morphology – Anatomy – Reproduction.

Introduction

The coast of Pakistan is rich in marine algal vegetation (SHAMEEL & TANAKA, 1992). Algae belonging to the order Gelidiales have been studied very poorly from the coast of Karachi (ANAND, 1943) and other coastal areas of the country (SHAMEEL & AFAQ-HUSAIN, 1987; SHAMEEL & al., 1989, 1996). At present the Gelidiaceae are little known from Pakistani waters. During a survey of the marine algal flora of the Karachi and Lasbela coasts several interesting red algae have been found (AFAQ-HUSAIN & SHAMEEL, 1991; AFAQ-HUSAIN & al., 1991). One of these, which appeared to be a new taxon, was studied critically and is described

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here as a new species of *Gelidium* Lamouroux. Its habitat, vegetative, anatomical and reproductive structures were investigated in detail.

Material and methods

Six populations growing at Manora, Buleji, Paradise Point, Pacha (Nathiagali), Naugaza Mazar and Cape Monze were studied during July-February 1985-1991. These spots lie 2-5 km apart from one another on a coastal belt near Karachi, Pakistan and experience more or less same environmental conditions. The plants were carefully scrapped alongwith their attachment systems. About 40-50 specimens were studied randomly from each population, 3-4 times a year in different months. The external morphology was examined from freshly collected specimens and cellular structure from those fixed in 4% formalin-seawater solution at the spot. Selected specimens were mounted on herbarium sheets for record, which are kept in the Herbarium of PCSIR, Karachi (CLH) and Seaweed Herbarium, MAH Qadri Biological Research Centre, University of Karachi (KUH-SW). The staining of different parts of the plants was done in 1% aniline blue for 24-48 h, either directly (for sectioning) or after treatment with 10% HCI for eight to ten days (for squashing to study the cells).

The cross sections (CS) and longitudinal sections (LS) were prepared by free hand cutting or with the help of a rotary microtome. The stained algal material was inserted in a cut made in a trimed potato chip, which was already mounted on a steel or wooden block. This whole mount was freezed in a deep freezer for 24 h and then run on a rotary microtome for sectioning. The sections were washed with distilled water to remove potato debris and mounted on slides. The temporary slides were prepared in distilled water or in a mixture of glycerine + acetic acid + distilled water (1:1:1, v/v) and permanent slides in Karo, a pancake syrup. Drawings were made with the help of a camera lucida.

Results

The six populations studied, exhibited same pattern of vegetation and range of characters, no difference of any kind was noticed between them. All populations were found very thin and the plants scattered in June-August and very rich in September-December become scanty in January onward. The plants are found growing on those sides of rocks and boulders which remain protected from direct wave action, in lower littoral zone. Each population consisted of plants of all range from June-February but frequency of the larger plants was very high in the months of September-December. Similarly sporophytic plants were found throughout study period but gametophytic plants could be collected in September-November. Following are the characters studied in detail of the new alga, *Gelidium usmanghanii* Afaq-Husain & Shameel.

Morphological characters

The plants are (2-)3-6(-7) cm tall, dark red in colour and grow in tufts. The thallus consists of a creeping system of loosely entangled, thin but tough thread-like structures, and a tuft of erect, flat, more or less papery but tough and cartilaginous fronds (Figs. 16-18). The creeping system is sparsely branched; its branches are 0.3-0.5 mm in diam., which are attached to substratum by minute pad-like structures and bear several upright axes at short intervals. Up to 1-3(-5) mm long proximal part of the uprights remains terete or cylindrical then turns into flat fronds. The terete part may give rise to secondary fronds or secondary creeping system. The flat fronds are 1-2.5(-3) mm wide and show spiral coiling along the long axis of the thallus. Their longitudinal middle part is plane but the peripheral part of either side is undulated. The apex, as seen in the microscope is broadly obtuse and may be either entire or slightly to deeply notched.

The branching is irregular and up to four orders, either from lateral margin or apex and in different planes. Usually several branch fronds arise from broken or shaded surface of remnant thallus. The branch fronds may be narrow or cuneate at base and their proximal part is terete and threadlike, whose length rarely reaches to 5 mm. Ramuli or pinnules of different shape and size develop sparingly to densely on the margin of the fronds specially near and around the apex (Figs. 16-18). These are narrow or cuneate at base and are unbranched to variously branched. The unbranched ones are oblong, clubshaped or nearly round, 0.6-1.5(-2.4) mm long and 0.4-0.75 (-1.1) mm broad (Fig. 12). The branched ones are 2-3 times branched and much elongated, they may reach a length of 5.0 mm and breadth of 1.6 mm (Figs. 9, 13-15). The ramuli may be non-fertile or fertile bearing spermatangia, carpogonia, cystocarps or tetrasporic sori. The naked fronds, which do not bear ramuli, are also common. Occasionally a ramulus may develop on flat surface of the thallus instead of margin.

Surface view of the thallus

In microscopic examination of surface of the thallus the epidermal cells appear angular, oblong to roundish, 3-7 µm long or broad. They are arranged randomly up to 4 µm apart from each other in mature thallus, but they are close and in semicircular rows around apical cell (Figs. 2 & 3). The latter is disk shaped, 5-9 µm broad and 2-5 µm thick. It is present either in the notch or emerged on the obtuse margin of apex of flat thallus (Figs. 2 & 3), but is always emergent in cylindrical terete thalli.

Anatomical features

In CS the outline of mature fronds appears as shown in Fig. 1. The fronds are thick in the middle (up to 600-800 µm wide, middle part is 180 µm thick) and the thickness gradually decreases towards margins. The latter appear conical but not pointed, in CS (Fig. 1). The cortical region is 20-25 µm thick, consisting of 3-4 layers of thin walled, pigmented cells, including epidermis. The epidermal cells may be roundish, oblong or angular, up to 8 µm long, 3-7 µm broad and are covered externally by 2-3 µm thick cuticle. After epidermis the cells become longer towards inside. The cells of third layer are measured up to 22 µm long and 9 µm broad. The CS and LS show that the respective cells are not uniform in shape, size and arrangement, except that they lie with their long axis parallel to the long axis of the thallus. However, in younger parts of thallus (close to apex) the epidermal cells appear more uniform in shape and arrangement; the thallus close to apex is 50 µm thick and its epidermal cells are elongated at right angle to the longitudinal axis of the thallus.

The medullary cells start appearing in 3rd cortical layer and the cortical/pigmented cells become scanty in the 4th layer. Rhizines are observed in abundance making a 30 µm broad layer below the cortex (Fig. 4). They are not present in the centre of medulla in the broader part of thallus, but in the narrow marginal parts the rhizines are present in the entire medulla. They are also not seen in the younger parts of thallus (close to apex). The rhizines are hyaline, refractive, cylindrical, up to 480 µm long and 2-4 µm broad; their distal ends are tapering and conical.

The medulla consists of cylindrical, thick walled colourless cells, which are up to 88 mm long and 20 µm broad (including colourless walls). The boundaries of their walls are usually not distinguishable but their central cytoplasmic portions get stained with aniline blue and can be seen easily. It is 2-8 µm broad but at the place of cytoplasmic connections with other cells it becomes up to 12 mm broad. The small and large cells are interspersed but the longest cells are present in the central medulla, they become shorter towards periphery. In creeping cylindrical branches the medullary cells are distributed randomly from below the epidermis to the central medulla and are up to 10 mm broad; small and large cells are interspersed. Rhizines are also present in peripheral region of these branches (Fig. 5).

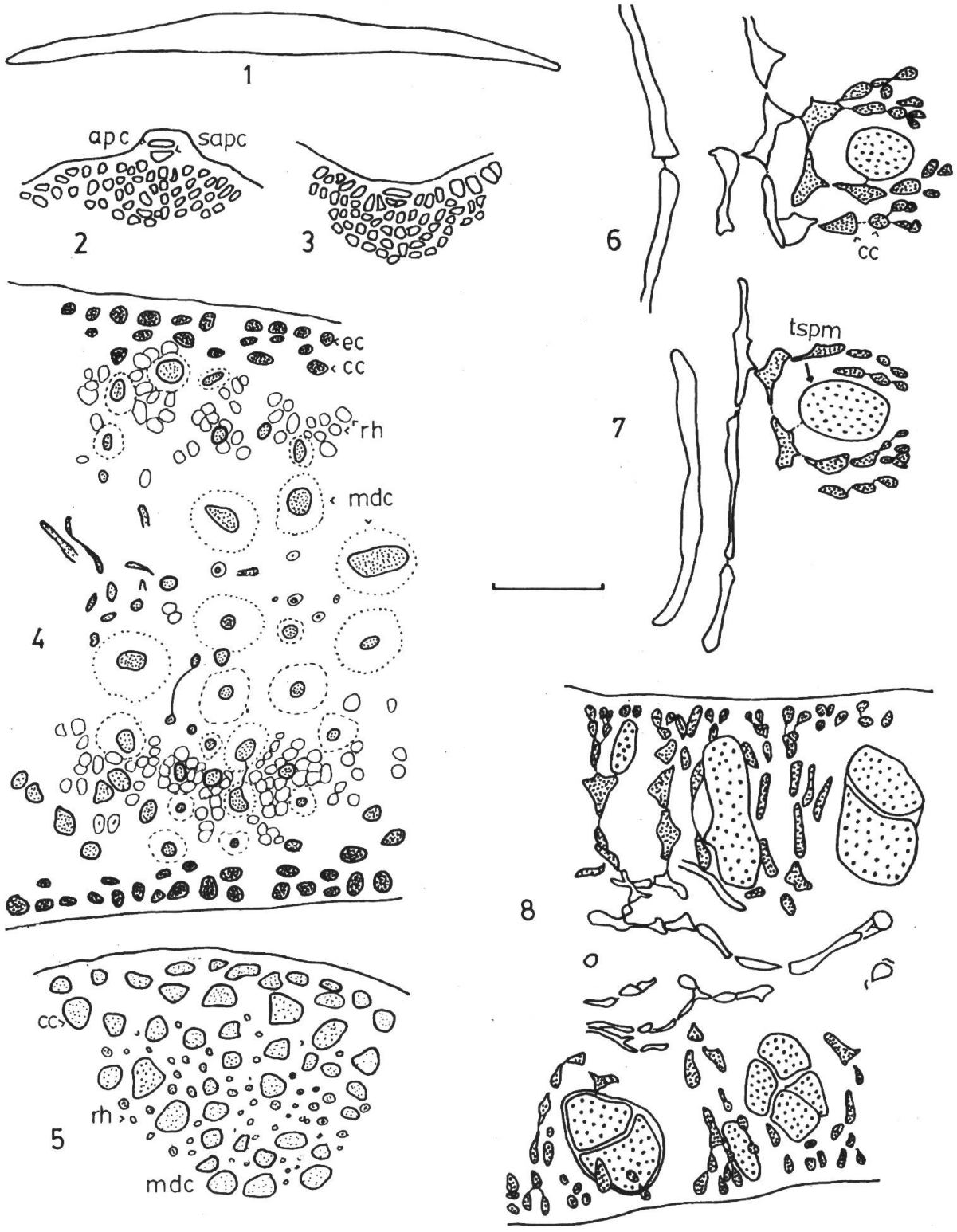

Fig. 1-8. – *Gelidium usmanghanii* Afaq-Husain & Shameel
1, outline of entire CS (cross section) of mature, flat thallus; **2 & 3,** surface view of apical portion of flat thalli; **4,** part of CS of mature flat thallus; **5,** part of CS of cylindrical (creeping thallus); **6-8,** LS (longitudinal section) of tetrasporangial stichidia (8, showing sporangia of different ages interspersed as well as first division parallel to surface; (**apc** = apical cell, **cc** = cortical cell, **ec** = epidermal cell, **mdc** = medullary cell, **rh** = rhizine, **sapc** = subapical cell, **tspm** = tetrasporangium; scale for figs.: **1** = 500 μm, **2-8** = 30 μm).

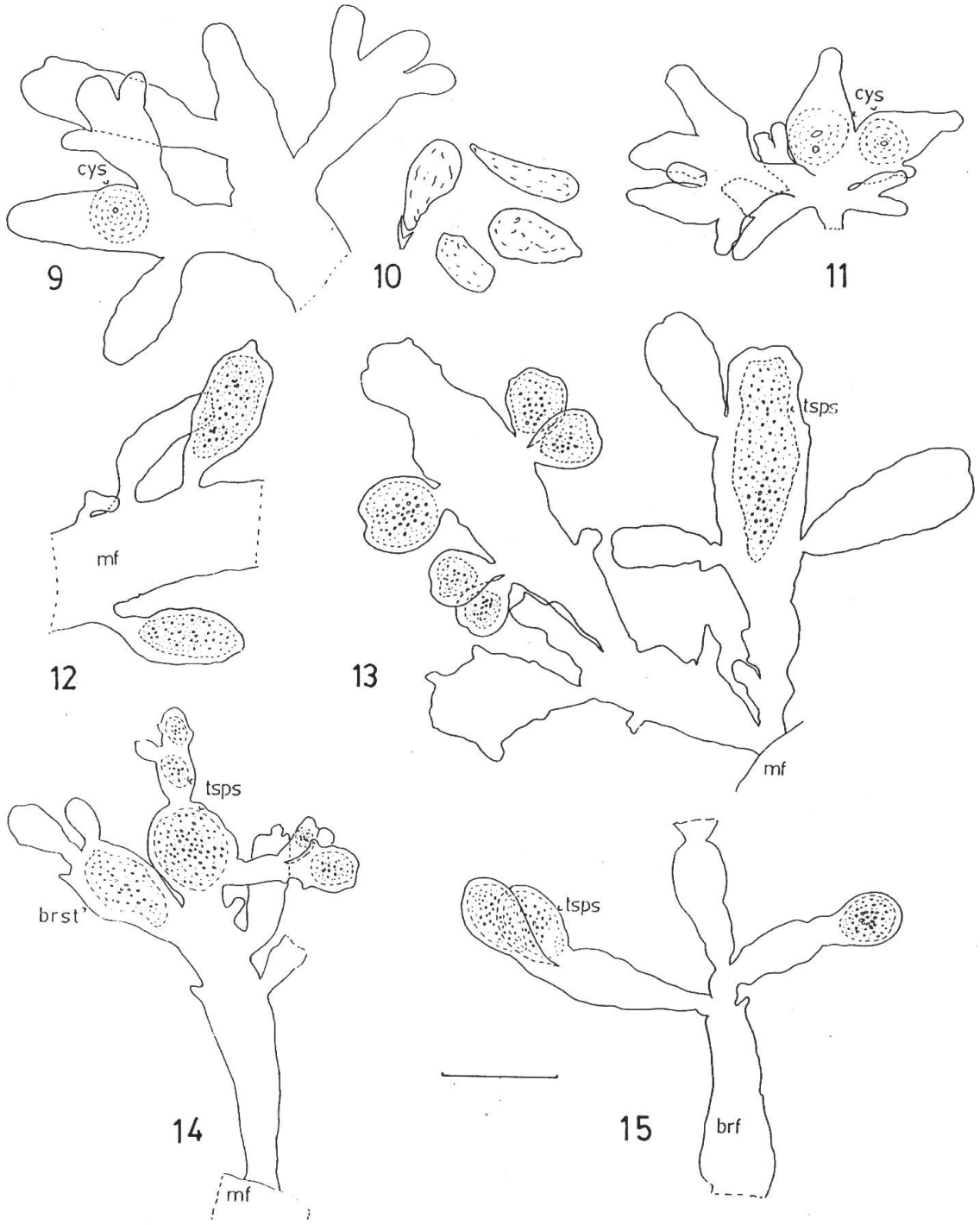

Fig. 9-15. – *Gelidium usmanghanii* Afaq-Husain & Shameel
9, cystocarpic ramuli with widely placed branching; **10,** carpospores, one with stalk-like remnant of carposporangia; **11,** cystocarpic ramuli with compact branching; **12,** small, oblong-elongate, unbranched pinnules arising from main frond; **13,** long, branched pinnule arising from main frond; **14,** branched stichidia showing tetrasporic sori one above the other; **15,** branch-frond bearing small, round or elongated tetrasporic sori terminally (**brf** = branch-frond, **brst** = branched stichidia, **cyst** = cystocarp, **mf** = main frond, **tsps** = tetrasporic sorus; scale for figs.: **9, 11-15** = 1000 µm, **10** = 60 µm).

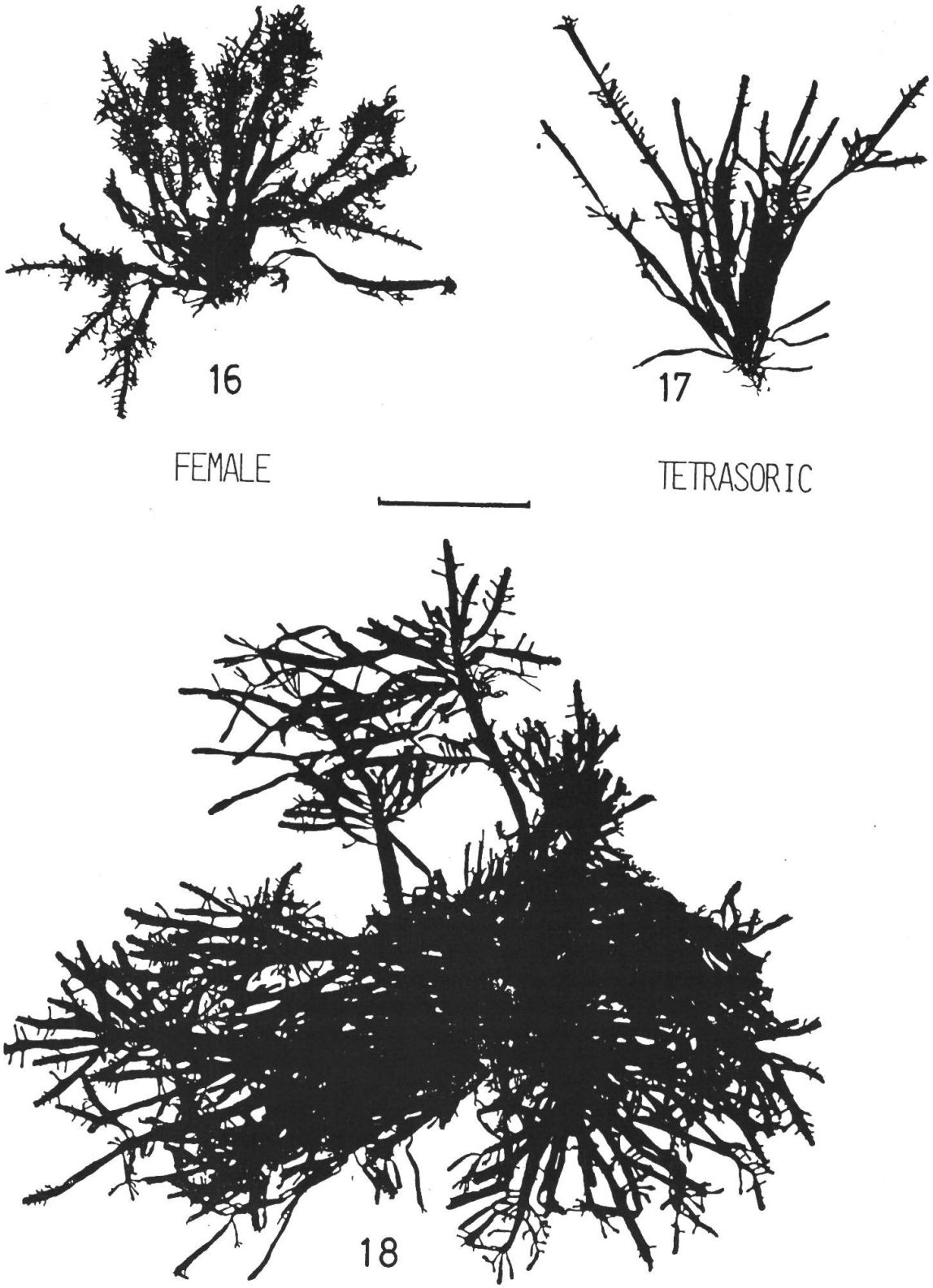

Fig. 16-18. – *Gelidium usmanghanii* Afaq-Husain & Shameel
16, female holotype with much branched and conjusted ramuli; **17,** tetrasporic holotype with sparse and unbranched ramuli; **18,** a tuft of many tetrasporic plants with branched and unbranched ramuli (scale for figs.: **16-18** = 20 mm).

Reproductive structures

Male, female and tetrasporic plants have been collected and examined under microscope critically. All three types are more or less similar in appearance, each can only be identified under microscope due to the presence of its reproductive bodies. In the population of this species the tetrasporic plants show much higher degree of dominance, they constitute about 70% of the population, while only a few male plants have been found.

Gametophytes. – The gametophytes are usually small. Their fronds are 2-3 cm long, 2.0-2.6 mm broad and usually not notched.

Spermatangia. – In male plants the spermatangia are produced by epidermal cells near the tip of young fronds or on stichidia and are visible under microscope as very small colourless cells on the surface. One or two spermatangial mother cells have been observed on the bearing cell. The spermatangial mother cells are narrow elongate, 4-6 μm long × 1-2.5 μm broad, each producing one spermatangium terminally by transverse division.

Carpogonia. – In female plants the carpogonia are observed on special branchlets or stichidia which are borne sparingly to densely on the margin of the fronds specially round the apex (Fig. 16). The cystocarpic stichidia may be unbranched (wich may be in the process of branching) to three times branched (branches turning to different planes) and are up to 5 mm long and less than 1 mm broad. The branches may be short and compact (Fig. 11) or long and widely spaced (Fig. 9), the ultimate branchlets taper to obtuse apex which is very rarely notched.

In the medulla an intercalary cell develops into a single-celled carpogonial branch. It consists of a broadly elongate basal portion, the carpogonium which is up to 12 μm long and 3-4 μm broad, and a distal trichogyne, which is about 40 μm long, curved, narrow below and broad at the apex. Near carpogonial branch the medullary cells are also found bearing chains of small round to oblong cells, up to 6 μm long and 2.5 μm broad, with dense cytoplasm, which may be the nurse cells.

Cystocarps. – The cystocarps are observed near the base of stichidial branchlets, as round to oblong protruding surfaces on both the sides of thallus. They are 500-750 μm long and 400-600 μm broad with 1-2 ostioles on each side (Figs. 9 & 11). In LS the cystocarps are clearly observed being bilocular.

Carposporangia. – The carposporangia are ovate to obpyriform, up to 65 μm long and 27 μm broad, very young to mature ones are found together. Stretched medullary cells in the form of thin filaments are observed running across the cystocarpic cavity, connecting the placenta to the wall of the cystocarp. These filaments consist of up to three cells, and are bifurcated at distal ends.

Carpospores. – Carpospores (released out under coverslip by slight pressure or teasing of the cystocarp) are oblong, elongate, tapering to base, (41-)50-60 μm long and (16-)21-24 μm broad (Fig. 10).

Sporophytes. – The tetrasporic plants are usually longer and broader than the gametophytic ones and their stichidial branches are also long and much branched. The fronds are usually 5-7 cm long and more than 2 mm broad, with apex broadly obtuse and slightly notched but smaller plants and deeply notched apices are also not uncommon.

Tetrasporangia. – The tetrasporangia are produced in sori-like patches on stichidial branches, which are highly variable in branching, shape and size (Figs. 12-15). Sometimes they are borne directly at the tip of fronds. The sorus appears 200-300 μm proximal to apex, it acquires the shape of the stichidia on which it is growing, leaving 80-270 μm broad sterile margin on either side. Thus it may be oblong to round or club-shaped when it develops on small stichidia (Figs. 12 & 13) or it may be elongated [1.0-2.0(-4.3) mm long and 0.5-1.2(-2.5) mm broad] when it develops at the tip of elongated stichidia (Fig. 13). New branches arise at the place of sorus and the stichidia continue to grow beyond the sorus and they further bear sori, which

appear one above the other (Fig. 14). In LS of tetrasporic stichidia many sporangia of different ages are seen interspersed, attached through cytoplasmic connections usually to 4th cell but sometimes to 3rd or 5th cell of the cortical filament (Figs. 6-8). In the sorus the cortex becomes 4-5 cells thick, and the filaments become curved along the developing sporangia. Rhizines are present in the stalk but absent in the sorus.

The mature tetrasporangia produce four tetraspores by cruciate division. The first division is parallel to surface of the thallus and second (in both halves) at right angle (Fig. 8). The periclinal divisions are observed without anticlinal divisions, but the latter ones are never seen without previous ones. In surface view the tetrasporangia of different ages are found scattered (not oriented in rows). Before division the tetrasporangia are 34 µm long × 24 µm broad and after division 45 µm long × 28-34 µm broad, ovate to oblong. After sheding of the tetraspores, the cells of the sorus degenerate and tear away, and new branches may arise from this portion.

General characters

Plants grow in tuft, up to 7 cm tall; creeping filaments cylindrical, 0.3-0.5 mm in diam.; the erect axes cylindrical at base (up to 5 mm long) abruptly turning into flat and spiral fronds, 1-3 mm wide, the marginal part undulated. Branching irregular, branch fronds and pinnules cuneate at the base. Unbranched to branched ramuli or pinnules of different shape and size develop sparingly to densely along the margin of fronds. Apical cell discoid, present in a depression between cortical lobes or protruding beyond the adjacent cortical cells. Epidermal cells in surface view rounded, angular with random orientation. In CS the erect fronds appear thick in the middle, tapering to either side into conico-obtuse ends. Rhizines abundant below the cortex and nearly absent in the centre of medulla in thicker part of flat thallus. Cystocarps on branched ramuli, bilocular with 1-2 ostioles on each side; filaments running across the cystocarpic cavity present. Carpospores oblongly elongate, tapering to base, up to 60 µm long × 24 µm broad. Tetrasporangia are produced in sori on unbranched to branched stichidia of different shape and size, or at the tip of fronds, on 3rd to 5th cell of the cortical filament, up to 45 µm long × 34 µm broad, oriented randomly in surface view.

Gelidium usmanghanii Afaq-Husain & Shameel, **spec. nova**

Plantulae crescunt unitae in basi; frons usque ad 7 cm alta, plana, in basi cuneata, spiraliter contorta, margine undulata. Ramuli simplices vel pinnati. Cellula apicalis discoidea prasens in depressione vel protrudens ultra cellulas corticales adjacentes. Cellulae superficiales et tetrasporangi disposita fortuito.

Holotype: H-9 PCSIR (Leg. *S. Afaq-Husain,* 26.9.1987) Buleji, Karachi, Pakistan (Figs. 16 & 17).

Isotypes: H-10 to H-15 PCSIR (Leg. *S. Afaq-Husain,* 26.9.1987) Buleji, Karachi, Pakistan (Fig. 18).

Other specimens examined: Manora (Leg. *S. Afaq-Husain & M. Shameel,* 29.12.1990, 28.1.1991); Buleji (Leg. *M. Shameel,* 15.9.1991); Paradise Point (Leg. *S. Afaq-Husain,* 29.9.1987, 21.12.1987, *M. Shameel,* 24.11.1991); Pacha, Nathiagali (Leg. *S. Afaq-Husain,* 5.2.1985, 13.11.1985, *M. Shameel,* 7.10.1991); Naugaza Mazar (Leg. *S. Afaq-Husain,* 15.10.1985, 23.7.1986); Lighthouse, Cape Monze (Leg. *S. Afaq-Husain,* 18.9.1985, 14.11.1985).

Habitat: Epilithic in lower littoral zone, usually on sites relatively protected from direct wave action but remaining wet with splashing water at low tides.

The new species, *Gelidium usmanghanii,* has been named after Prof. Dr. K. Usmanghani, who has made several contributions on the bioactivity and phycochemistry of the marine algae of Pakistan including those of Rhodophyta.

Discussion

The generic characters for *Gelidium* Lamouroux as described by RENFREW & al. (1989) are: (a) presence of rhizines, (b) mature cystocarps bilocular and (c) apical and lateral initials morphologically distinct; lateral initials not in cortical indentations. Our plants clearly exhibit the first 2 characters but the last one is not exhibited. The validity of the last character for *Gelidium* is not correct. RENFREW & al. (1989) based this character to the findings of RODRIGUES & SANTELICES (1987) and observed themselves in all the three species (*G. coulteri* Harvey, *G. purpurascens* Gardner, *G. vagum* Okamura) they studied. But FELICINI & PERRONE (1994) atach no taxonomic value to the form of apical cell, whether projected or present in depression, because it is not due to genetic factor, it is simply related to different rates of growth between apical and neighbouring cells.

Besides bilocular cystocarps, thin-celled filaments traversing the cavity from central placenta to the wall of the cystocarp and unordered orientation of surface cells as well as tetrasporangia are the characters of the genus *Gelidium* (AKATSUKA, 1986; HOMMERSAND & FREDERICQ, 1988; RENFREW & al., 1989; STEWART, 1992; which are clearly observed in the present plants. Thus the placement of these plants in the genus *Gelidium* Lamouroux is beyond doubt.

The present populations differ, from other species of *Gelidium,* which bear flat erect fronds, in external appearance as well as some internal structures. The small size of plants, flat shape of erect fronds which are thick in the middle, spiral coiling along the long axis of the thallus, undulations on lateral parts of flat thalli are distinguishing features of *G. usmanghanii.* Moreover, much branched and conjusted growth of proliferations (pinnules or stichidia) on the margins of flat fronds and irregular branching of thallus itself, together with the above mentioned characters distinguish this species from other gelidiaceous taxa. The present plants are, therefore, treated as a new specific taxon of *Gelidium* in this work.

Seven species of *Gelidium* [*G. corneum* (Hudson) Lamouroux, *G. crinale* (Turner) Lamouroux, *G. heteroplatos* Børgesen, *G. micropterum* Kützing, *G. pulvinatum* (Kützing) Turner, *G. pusillum* (Stackhouse) Le Jolis and *G. rigidum* J. Agardh] have been reported from Sri Lanka, India and Iran, the countries which are near or adjoining the coast of Pakistan (BØRGENSEN, 1933, 1934a, b, 1935, 1936, 1937, 1938, 1939; DURAIRATNAM, 1961; DIXIT, 1964; KRISHNAMURTHY & JOSHI, 1970). These species are, therefore, expected to occur in the region of study but only *G. pusillum* is so far reported from here (ANAND, 1943; SHAMEEL & AFAQ-HUSAIN, 1987; SHAMEEL & al., 1989, 1996; SHAMEEL & TANAKA, 1992). These species are circumscribed inadequately and misidentified in different areas by different workers as pointed out by DIXON & IRVINE (1977), STEWART & NORRIS (1981), RENFREW & al. (1989), FREDRIKSEN & al. (1994), FRESHWATER & RUENESS (1994) and SANTELICES (1994).

The present account is the first attempt to study a gelidiaceous taxon, circumscribing morphological, anatomical and reproductive characters, defining it clearly, from this area. It is, therefore, difficult to compare the present studies with previous ones. However, among the above seven species, three (*G. corneum, G. heteroplatos* and *G. micropterum*) are flat and will be discussed here. BØRGENSEN (1938) proposed that the species cited as *G. corneum* in his work (1935, 1936, 1937) should be treated as *G. micropterum* Kützing because *G. corneum* was not identifiable and the specimens from India and Sri Lanka agree very well with Kützing's figure of *G. micropterum* (Tab. Phycol. Vol. 18, pp. 21, Pl. 59). This shows that both the species are more or less identical. Our plants do not resemble Kützing's figure of *G. corneum* or *G. micropterum* (KÜTZING, 1868) or the Korean plants (LEE, 1988). The new species also does not resemble with *G. heteroplatos* because the latter is clearly distinguishable by alternately compressed and terete thallus, which is less than 1 mm broad.

Other flat species described from different parts of the world are:

1. *G. biserratum* Børgesen,
2. *G. chilense* (Montagne) Santelices & Montalva,
3. *G. filicenum* Bory,
4. *G. johnstonii* Setchell & Gardner,
5. *G. latifolium* (Greville) Bornet & Thuret,
6. *G. lingulatum* Kützing,
7. *G. sesquipedale* (Turner) Thuret,
8. *G. subcostatum* Okamura,
9. *G. vagum* Okamura.

Except *G. chilense* all other species are much longer than the present plants and do not resemble with the new species in habit. *Gelidium chilense* is a small species like *G. usmanghanii* but it differs in having shield-shaped tretrasporangial stichidia and serrate margin of the thallus and the stichidia both (SANTELICES & MONTALVA, 1983; SANTELICES & STEWART, 1985). Moreover, in *G. chilense* the tip of frond is flattened and truncate (RODRIGUEZ & SANTELICES, 1987), but it is obtuse in the present species. It is, therefore, concluded that the present species is a new specific taxon of *Gelidium.*

In the end the growing behaviour of the present species may be emphasized which may further separate it from other allied species. The populations of *G. usmanghanii* prefer to grow hanging from margins and vertical sides of rocks and boulders as well as the rim of pools which become visible at low tides. They are thickly populated on margins and less so on vertical sides but negligible or nil on horizontal surface. The vegetation is only found in opposite direction of wave action.

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