

FURTHER STUDIES ON *GELIDIUM* (RHODOPHYTA) FROM THE COAST OF PAKISTAN

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Abstract

The circumscription of *Gelidium pusillum* (Stackhouse) Le Jolis has been discussed and a new taxon *G. pusillum* var. *pakistanicum* Afaq-Husain et Shameel is described. The vegetative, anatomical and reproductive structures of the Pakistani populations of this species have been investigated in detail. The new variety showed distinctive characters as turf or cushion forming habit, the primary erect fronds being teretocompressed below and becoming flat in the upper half, plants up to 11 mm in length appearing distinctly segmented under microscope, random arrangement of surface cells as well as of tetrasporangia borne on stichidia-like structures as well as on the tips of branch fronds, presence of rhizines in abundance and sexual plants not being present. This species has also been compared with *G. usmanghanii* Afaq-Husain et Shameel.

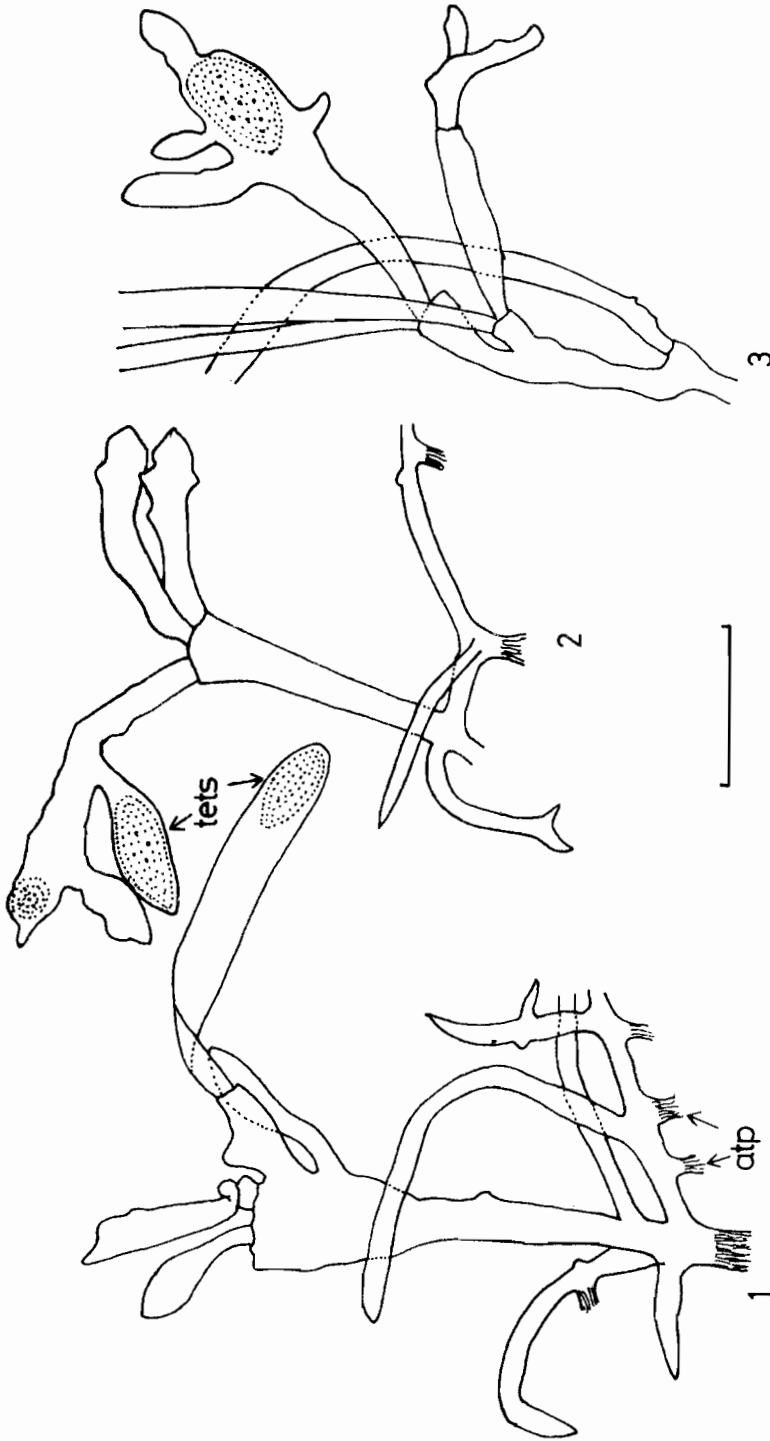
Introduction

The genus *Gelidium* Lamouroux has been reported from Karachi, Lasbela, Makran and other coastal areas of Pakistan but without any taxonomic detail (Anand, 1943; Shameel & Afaq-Husain, 1987; Shameel *et al.*, 1989, 1996; Shameel & Tanaka, 1992). Recently a new species, *Gelidium usmanghanii* Afaq-Husain et Shameel has been described from Pakistani waters and *G. pusillum* (Stackhouse) Le Jolis was taxonomically enumerated (Afaq-Husain & Shameel, 1996, 1997). However, the identification of gelidiaceous taxa is very difficult due to a high degree of intraspecific variations (Feldmann & Hamel 1936; Dixon & Irvine 1977; Fredriksen *et al.*, 1994). In the present work the Pakistani populations of *G. pusillum* were found to differ from the original description of Stackhouse (1795) and a recent one by Fredriksen *et al.* (1994) in few respects. After comparison with other varieties and with *G. usmanghanii* these populations have been described as a new variety.

Materials and Methods

Atleast 100-200 specimens of algae were collected during each survey as epilithon from lower littoral rocks at Manora, Buleji, Paradise Point, Nathiagali, Naugaza Mazar and Cape Monze near Karachi, Gadani and Sonmiani at the coastal areas of Lasbela, and Gwader and Jiwani at the coast of Makran, Pakistan during September 1985 and December 1996. Some specimens were fixed in 4% formalin-seawater solution and others mounted on herbarium sheets which are kept in the Herbarium of PCSIR,

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Figs. 1-3. *Gelidium pusillum* var. *pakistanicum* Afaq-Husain et Shameel: Plants showing habit. (atp = attaching pads, tats = tetrasporic sorus; Scale = 1.2 mm).

Karachi (CLH) and Seaweed Herbarium, MAH Qadri Biological Research Centre, University of Karachi (KUH-SW). The staining of whole plants or parts of them was carried out in 1% aniline blue for 24-72 h and sectioning was done either by free hand cutting or by an ordinary rotary microtome. The other techniques were the same as described previously (Afaq-Husain & Shameel, 1996).

Results.

In Pakistan the genus *Gelidium* is represented by *G. pusillum* and *G. usmanghanii* and both of them comprise small thalli, but species with large plants do not seem to occur in this area. They may be distinguished as follows:

Plants grow in small turfs, up to 11 mm long ----- *G. pusillum*

Plants grow in tufts, 2-7 cm tall ----- *G. usmanghanii*

1. *GELIDIUM PUSILLUM* (STACKHOUSE 1795) LE JOLIS 1863 VAR. *PAKISTANICUM* AFAQ-HUSAIN ET SHAMEEL VAR. NOV.

Morphological characters: Plants grow in small, dense, low turfs or cushions of about 5 cm diam., or larger due to close development of 2 or more cushions. Their colour is brownish red to blackish maroon. The cushions are up to 5 mm thick and consist of creeping and erect systems. The creeping system is thread-like, cylindrical, 0.1-0.3 mm in diam., irregularly branched with conical apices and attached to the substratum by small attaching pads at small intervals on lower side, up to 300 μ m long and as broad as



Fig. 3a. *Gelidium pusillum pakisticum* var. nov.: Holotype (X70).

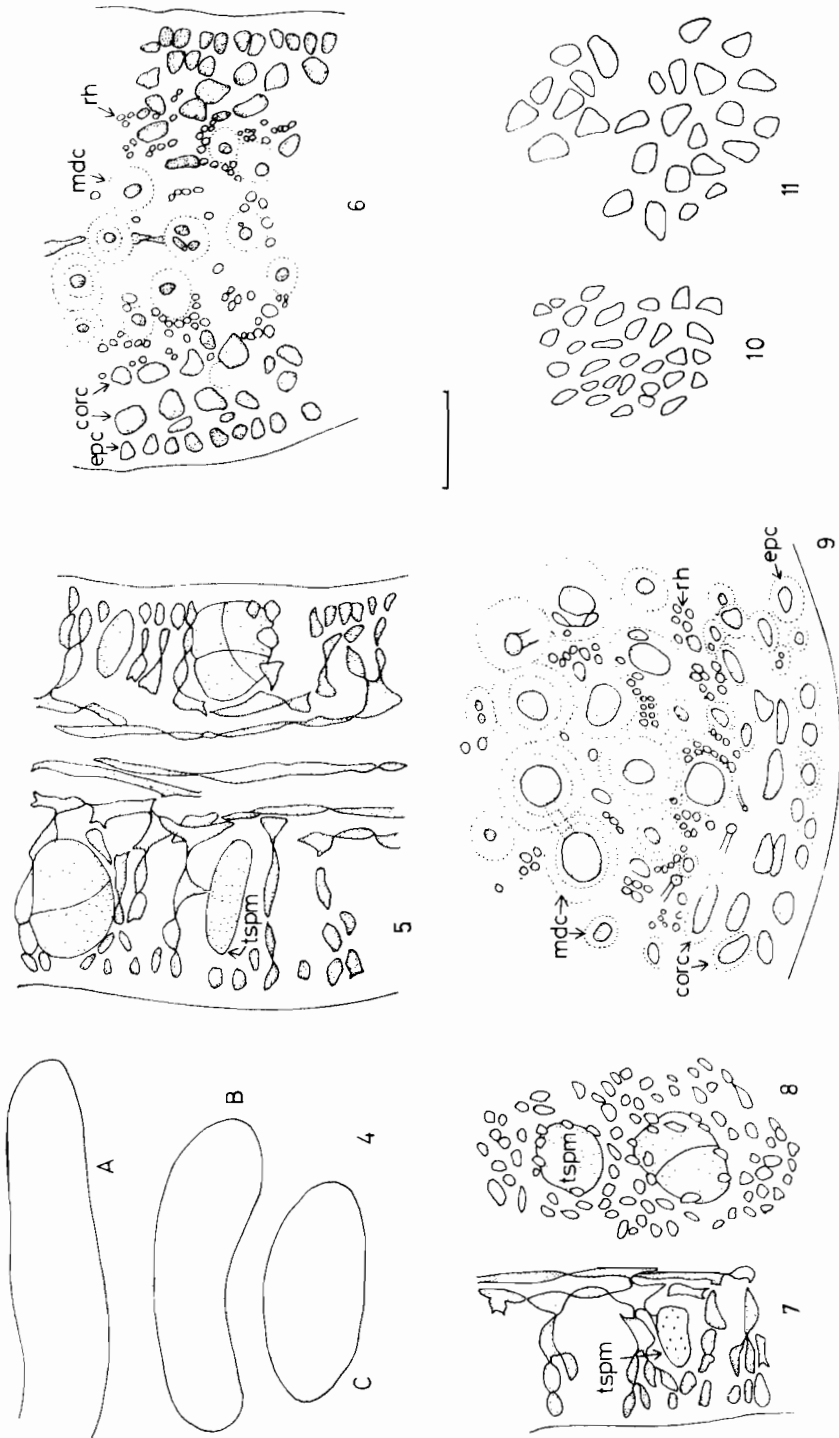
the creeping axes and made up of elongated cells (Figs. 1-3). The creeping axes give rise to erect fronds (usually at places opposite to attaching pads), which are up to 11 mm long, intermingled into small cushions. The erect ones arise as cylindrical branches with conical apices gradually turning to terete-compressed, up to 0.25 mm thick and finally become flat, up to 0.6 mm wide x 0.1 mm thick (Fig. 4).

Surface view of the thallus: In surface view of mature parts of thalli the cells appear roundish, angular or elongated and are oriented randomly. In creeping axes the cells appear larger (up to 17 μm long x 12 μm broad) than those of erect fronds (up to 5 (-10) μm in diameter in terete thalli and 9 μm in flat, (Figs. 8, 10 & 11). The apical cell appears discoid to slightly conical proximally (depending upon the condition of division), up to 2-5 μm long x 4-7 μm broad and is always emergent (not found in depression).

Anatomical features: Cross sections (C.S.) of the basal, middle and upper parts of erect fronds are circular, ellipsoid (up to 240 μm thick) and linear (up to 108 μm thick) in outline (Fig. 4 A-C). The C.S. of the younger fronds are also ellipsoid in outline showing that the upright axes arise as cylindrical branches, which gradually become flat distally. The cortex is 2-3 cell layers thick; epidermal cells 5-7 μm in diameter, usually slightly longer than broad, and arranged with their long axis at right angle to surface; the inner cortical cells become up to 9 μm in diameter. The cells below the cortex become broader and longer towards interior but small and large cells are interspersed and they lie along the long axis of the thallus. The medullary cells are up to 148 μm long x 16 μm broad, round to oblong in C.S. and their walls are thick and colourless, when stained with aniline blue, the cytoplasm appears irregular in diameter which is up to 6 μm long (Fig. 6). Rhizines are present in large number below the cortex, decreasing towards interior, and are up to 360 μm long x 2-5 μm broad with tapering conical ends (Fig. 9).

Reproductive structures: Only tetrasporic plants were found (Figs. 1-3). The tetrasporic sori develop at the tip of flat fronds (Fig. 1) or in stichidia-like structures which arise from lateral margin of flat fronds unbranched and ellipsoid in outline (Fig. 2). The sori are 350-620 μm long at the tip of frond and as broad as the thallus with a narrow, 10-20 μm broad sterile margin, and slightly thicker than vegetative axes. The tetrasporangia of different ages are interspersed, easily observed in surface view (Fig. 8), and measure up to 29 μm long x 21 μm broad. In longitudinal section (L.S.) of tetrasporic sori, the cortical filaments are 3-4 cells long, cells being elongate to 12 μm x 5 μm broad; the tetrasporangial initial cell arises from 3rd or 4th cell of the cortical filaments, it elongates first and then broadens becoming up to 43 μm long x 31 μm broad. The tetrasporangial initial cell undergoes meiosis consequently forming the tetrads (Figs. 5 & 7). The first division is periclinal followed by anticlinal division which is independent of each other in each half.

General characters: Plants grow in small turfs, caespitose, up to 11 mm long. Creeping filaments cylindrical, up to 0.3 mm thick, consisting of broader cells than in erect fronds. Erect axes cylindrical at base, gradually turning to compress and then flat in the upper part of the segment, cylindrical part up to 0.24 mm thick and flat part up to 0.6 mm broad and 0.1 mm thick. Branches produced mostly by regeneration of fronds at shed or broken ends. The apical cell conico-discoid, protruding beyond the adjacent



Figs. 4-11 *Gelidium pusillum* var. *pakistanicum*:
 4. A-C. Outline of cross section (C.S.) of erect fronds, 5 & 7. Longitudinal sections (L.S.) of tetrasporic sorus, 6. A part of C.S. of terete, erect frond showing cellular details, 8. Surface view of tetrasporic sorus, 9. A part of C.S. of creeping frond showing cellular details, 10. Cells in surface view of terete, erect frond, 11. Cells in surface view of creeping frond. (corc = cortical cell, epc = epidermal cell, mdc = medullary cell, rh = rhizine, tspm = tetrasporangium; Scale for figs. : 4 = 120 μ m, 5-11 = 30 μ m).

cortical cells, the surface cells roundish-angular with random orientation in the middle part of erect fronds. The tetrasporangia occur in sori at the tip of erect fronds or in stichidia-like structures, oriented randomly.

Diagnosis: *Gelidium pusillum* var. *pakistanicum* Afaq-Husain et Shameel var. *nova*: *Plantulae ad 11 mm longa, faciunt vel humum herbosum. Frondes erecti, cylindricales in basi, gradatim fiunt plani in partem mediam superiorem, usque ad 0.24 mm lati. Cellula apicalis conico-discoidea, ultra cellulas conicales adjacentes protrudens. Cellulae superficiales (in frondis medio), quasi rotundae, sine ordine orientatae. Tetrasporangia etiam sine ordine orientata.*

Holotype: H-21 PCSIR (*Leg.* S. Afaq-Husain 27-7-1986) Gadani, Karachi, Pakistan (Fig. 3a).

Isotypes: H-22 - 24 PCSIR (*Leg.* S. Afaq-Husain 27-7-1986) Gadani, Karachi.

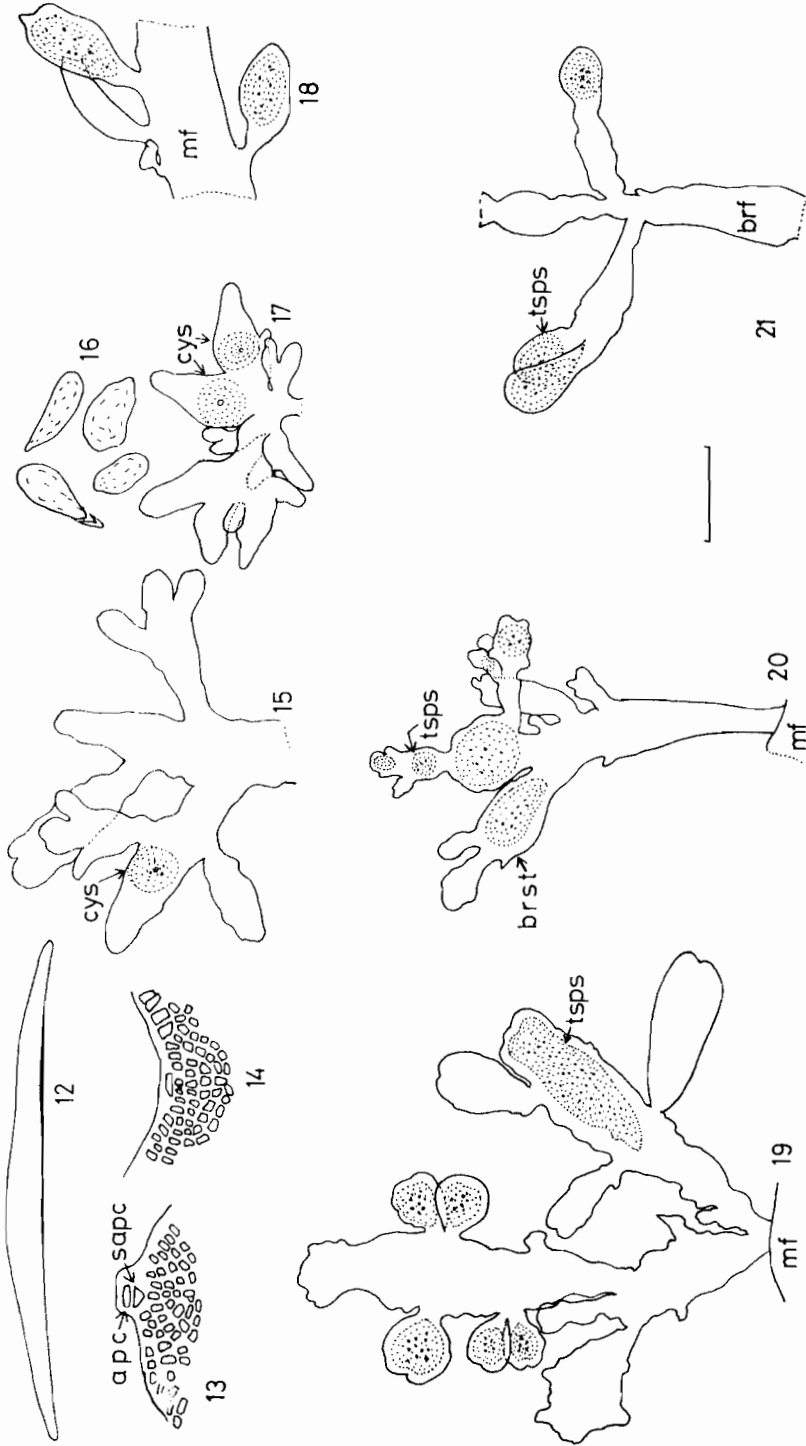
Other specimens examined: Buleji (*Leg.* M. Shameel 18-10-1990, 8-12-1993, 24-4-1996, 4-12-1996), Paradise Point (*Leg.* S. Afaq-Husain 20-12-1987, 12-4-1996, M. Shameel 15-10-1988), Cape Monze (*Leg.* S. Afaq-Husain 8-9-1985, M. Shameel 23-11-1989), Sonmiani (*Leg.* S. Afaq-Husain 17-9-1985, M. Shameel 21-12-1996), Gwader (*Leg.* M. Shameel 23-12-1996), Jiwani (*Leg.* M. Shameel 26-12-1996).

Habitat: Epilithic on lower littoral rocks.

2. *GELIDIUM USMANGHANII* AFAQ-HUSAIN ET SHAMEEL 1996

Plants grow in tufts, up to 7 cm tall, creeping filaments cylindrical, 0.3-0.5 mm in diameter; the erect axes cylindrical at base (up to 5 mm long) abruptly turning into flat (Fig. 12) 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 (Fig. 14) or protruding beyond the adjacent cortical cells (Fig. 13). Epidermal cells in surface view roundish, angular with random orientation. In C.S. the erect fronds appear thick in the middle, tapering to either side into conico-obtuse ends (Fig. 12). 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 (Figs. 15 & 17); filaments running across the cystocarpic cavity present. Carpospores oblongly elongate, tapering to base, up to 60 μm long x 24 μm broad (Fig. 16). Tetrasporangia are produced in sori on unbranched to branched stichidia of different shape and size (Figs. 18-20), or at the tip of fronds (Fig. 21), on 3rd to 5th cell of the cortical filament, up to 45 μm long x 34 μm broad, oriented randomly in surface view.

Epilithic in lower littoral zone, usually on sites relatively protected from direct wave action but remaining wet with splashing water at low tides. Specimens examined were collected from: Manora (*Leg.* S. Afaq-Husain 29-12-1990, M. Shameel 28-1-1991), Buleji (*Leg.* S. Afaq-Husain 26-9-1987, M. Shameel 15-9-1991, 19-12-1996), Paradise Point (*Leg.* S. Afaq-Husain 29-9-1987, 21-12-1987, M. Shameel 24-11-1991, 19-12-1996), Pacha, Nathiagali (*Leg.* S. Afaq-Husain 5-7-1985, 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).



Figs. 12-21. *Gelidium usmanghanii*. Afaq-Husain et Shameel:

12. Outline of entire C.S. of mature, flat thallus, 13 & 14. Surface view of apical portion of flat thalli.

15. Cystocarpic ramuli with widely placed branching, 16. Carpospores, one with stalk-like remnant of carposporangia. 17. Cystocarpic ramuli with compact branching, 18. Small, oblong-elongate, unbranched pinnules arising from main front, 19. Long, branched pinnule arising from main front.

20. Branched stichidia showing tetrasporic sori one above the other, 21. Branch-front bearing small round or elongated tetrasporic sori terminally.

(apc = apical cell, brf = branch-front, brst = branched stichidia, cyst = cystocarp, mf = main front, sapc = subapical cell, tsps = tetrasporic sorus; Scale for figs. : 12 = 500 μ m, 13 & 14 = 30 μ m, 15, 17-21 = 1000 μ m, 16 = 60 μ m).

Discussion

The present work has revealed that on the coast of Pakistan the genus *Gelidium* is represented by two species only viz., *G. pusillum* and *G. usmanghanii*, both being small in size. This genus yields the well known phycocolloid agar and is, therefore, commercially very important but the commercial exploitation of the two Pakistani species needs investigation although *G. pusillum* is too small and so scanty that it cannot be employed for agar extraction and *G. usmanghanii* has a very poor yield of this phycocolloid (Afaq-Husain 1992a).

The species of *Gelidium* exhibit a wide range of variation in morphological characters and produce confusion in identification. Moreover, several species were not properly circumscribed due to which earlier workers had misidentified them or wrongly created specific or intraspecific taxa (Stewart & Norris, 1981; Santelices & Montalva, 1983; Renfrew *et al.*, 1989). Subsequently new parameters such as surface cell morphology and patterns of apical cell structure combined with vegetative and reproductive structures were emphasised for the taxonomic analysis of gelidiaceous taxa (Akatsuka, 1986; Rodriguez & Santelices, 1987; Renfrew *et al.*, 1989). Later on these parameters also proved unreliable because they vary with ontogeny and environment, having no genetic basis (Rodriguez & Santelices, 1988; Santelices, 1990; Stewart, 1992; Felicini & Perrone, 1994). Thus *G. pusillum* remained a confused species for a long time but now it has been defined satisfactorily with the help of modern biosystematic techniques (Fredriksen *et al.*, 1994; Freshwater & Rueness, 1994).

Initially various other species have been incorporated in *G. pusillum*, as pointed out by several workers (Dixon & Irvine 1977; Stewart & Norris, 1981; Renfrew *et al.*, 1989; Hatta & Reine, 1991). Less than 1 cm to 15 cm tall plants were included in it, which might be cylindrical, terete, compressed or flat, and simple to much branched (Abbott & Hollenberg 1976; Dixon & Irvine, 1977; Stewart & Norris, 1981; Lee, 1988). Renfrew *et al.* (1989) expressed their views about *G. pusillum* as: "The name seems to have been applied commonly to any small turf-forming, compressed to flattened gelidiaceous plant. The taxon clearly needs revision on global scale, beginning with a thorough study of the type material". Recently this species has been restudied and defined satisfactorily with the help of modern techniques of rbcL nucleotide sequence analysis (Freshwater & Rueness, 1994) and interfertility and culture studies *in vitro* (Fredriksen *et al.*, 1994), thus leaving no ambiguity in the identification of *G. pusillum*.

Freshwater and Rueness (1994) pointed out that all populations with small plants might not be *G. pusillum*. They reported that "The name '*Gelidium pusillum*' has commonly been used to refer to any small *Gelidium* species found in exposed habitats in the intertidal zone throughout the world. Examination of several such taxa has so far not revealed any specimen related to *G. pusillum* from Europe". They suggested that there is a need to investigate the morphology of each species critically in comparison with other kinds of observations including molecular data. During our studies the morphological and anatomical studies have been done critically which show that Pakistani populations differ from *G. pusillum*, only to the extent to form a new varietal entity.

As compared with the descriptions of Stackhouse (1795) and Fredriksen *et al.*, (1994), the Pakistani populations of *G. pusillum* differ in the following characters:

Size of plants: According to Fredriksen *et al.*, (1994: 464-465, Figs. 4 & 8) their plants are 5-30 mm long and up to 1 mm broad, but in Pakistan they are up to 11 mm long and 0.6 mm broad.

Striations: There are no striations on any part of fronds of the present populations which are described to be present on European plants by Fredriksen *et al.*, (1994), however longitudinal chains of medullary cells have been observed inside the fronds when examined under microscope.

Shape of branches: Stackhouse (1795) and Fredriksen *et al.*, (1994) described the branches of their plants to be rounded or ovoid but in the present case they are distinctly conical. However, the tips of flat branch fronds become obtuse in due course of development.

Mode of branching: Primarily the branching is lateral and does not appear forked as is described by Stackhouse (1795) and Fredriksen *et al.*, (1994). However, terminal branching occurs only from broken surface of remnant fronds and may appear forked; the emergence of new branches from broken surface is a common way of branching in the present plants and due to which they appear jointed.

Shape of fronds: According to Stackhouse (1795) and Fredriksen *et al.*, (1994: 464, Fig. 1) the erect fronds are spatulate and produce branches in the form of forks or horns which appear constricted or stalked at base. In the present populations the plants consist of both terete and flat fronds, the terete branches usually turn into flat thalli gradually and do not appear constricted or stalked at base.

Texture and colour: Stackhouse (1795) described *G. pusillum* to be rigid and horny in substance and pale red in colour when seen in light. The present plants are no doubt tough in texture but not rigid and horny, and their colour is blackish maroon, never appearing pale.

Habitat: Fredriksen *et al.*, (1994) have clearly characterised *G. pusillum* to grow on open rocks in upper and mid littoral, but Pakistani populations are only found in lower littoral zone probably to protect themselves from heat and sun.

The above mentioned variations are enough to treat the Pakistani populations of *G. pusillum* as a new variety. These populations were found to differ in general characters from *G. pusillum* var. *cylindricum* Taylor and *G. pusillum* var. *pacificum* Taylor from China (Santelices, 1988) and Korea (Lee, 1994). They were also compared with *G. pusillum* var. *pulvinatum* (C. Agardh) J. Feldmann from Korea (Lee & Kim, 1995) and exhibited remarkable differences.

From ecological point of view it is interesting to note that out of 7 species of *Gelidium* viz., *G. corneum* (Hudson) Lamouroux, *G. crinale* (Turner) Lamouroux, *G. heteroplatos* Børgesen, *G. micropterum* Kützinger, *G. pulvinatum* (Kützinger) Thuret, *G. pusillum* and *G. rigidum* J. Agardh, which occur on the adjoining coasts of India and Iran (Børgesen, 1933, 1935, 1939) as well as Sri Lanka (Durairatnam, 1961), only *G. pusillum* has been found to grow along the coast of Pakistan (Shameel & Tanaka, 1992). Although all these countries border Arabian Sea in the Indian Ocean, but the absence of other species on Pakistani coast is surprising. Moreover, the allied genera *Gelidiella* Feldmann et Hamel and *Pterocladia* J. Agardh, which also occur on the coast

Table 1. Comparative features of the two species of *Gelidium* from Pakistan.

Characters	<i>G. pusillum</i> var. <i>pakistanicum</i>	<i>G. usmanghanii</i>
Plant height	up to 11 mm	up to 7 cm
Habit: turf-making/ not-turf-making/	turf-making	present in tufts but not turf-making
Erect thallus: terete/ flat	terete below, flat above	throughout flat
Outline of T.S. of erect frond	circular, ellipsoid to linear	elongated, broad in the middle, tapering to margins
Width of flat frond	0.6 mm	1-3 mm
Thickness of flat frond	0.10 mm	0.18 mm
Creeping system	cylindrical, 0.33 mm broad	cylindrical, 0.5 mm broad
Apical cell: shape and position	conico-discoïd, 5 μ m long x 7 μ m broad, protruding beyond cortical cells	discoïd, 2 μ m long x 9 μ m broad, present in depressions or protruding beyond cortical cells
Surface cells of erect thallus, and their orientation	cells in the mid frond roundish, 5-7 μ m broad, oriented randomly; cells at the base elongated, orien- ted across the longitudinal axis	roundish-angular, 3-7 μ m broad, oriented randomly
Cortex	2-3 cell layers thick	3-4 cell layers thick
Rhizines	abundant below the cortex	abundant below the cortex
Ramuli	unbranched, not dense	branched or unbranched, sparse to dense
Tetrasporangia: orien- tation	up to 43 x 31 μ m, oriented randomly	up to 45 x 34 μ m, oriented randomly
Cystocarp	not present	0.7 mm broad, on branched ramuli
Carpospores	not present	elongated, up to 60 x 24 μ m

of India (Krishnamurthy & Joshi, 1970), have not been recorded so far from Pakistani waters.

Gelidium usmanghanii may be easily distinguished from other species by its flat, papery fronds, which show coiling along the long axis and bear undulations towards margin. Moreover, irregularly branched and thickly populated ramuli on margin of the fronds constitute another distinguishing character of this species (Afaq-Husain & Shameel, 1996). The two Pakistani species of *Gelidium* differ remarkably from one another (Table I). Although, both of them are epilithic on lower littoral rocks, *G. pusillum* prefers exposed and flat rocks while *G. usmanghanii* grows hanging from margins and vertical sides of protected rocks and boulders as well as the rim of pools

which become visible at low tides. The latter populations are found away from wave action. Due to scarcity of the material for chemical purposes the chemical constituents of *G. pusillum* can not be compared with those of *G. usmanghanii* (Afaq-Husain 1992b).

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