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FURTHER INVESTIGATIONS ON THE RED ALGA *MELANOTHAMNUS AFAQHUSAINII* (CERAMIALES) FROM THE COAST OF PAKISTAN

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Abstract

External characters, anatomical features and characteristics of propagules of the red alga *Melanothamnus afaqhusainii* Shameel is described and compared with the Somalian and Yemeni populations of *M. somalensis* Bornet *et* Falkenberg. *M. afaqhusainii* differs from *M. somalensis* by its larger size, larger propagules containing more tiers of cells and the thicker cortication of the axis. *M. afaqhusainii* also differs from Somalian populations of the type species, *M. somalensis*, in having conical and spindle-shaped propagules and from Yemeni populations of *M. somalensis* in habit and in the production of the propagules on dwarf shoots (*Polysiphonia*-like filaments) only.

Introduction

Melanothamnus Bornet *et* Falkenberg (Rhodophyta, Ceramiales, Rhodomelaceae) was until recently considered to be a monotypic genus (Falkenberg, 1901). The only species, *M. somalensis* Bornet *et* Falkenberg (type locality: Somalia) has been reported from Somalia, Yemen and Oman (Wynne & Banaimoon, 1990). The generic characters as described by Kylin (1956) are: dark coloured, cylindrical, up to 36 cm long, radially branched thalli; tips of shoots with distichous branch arrangement; shoots bear long, obliquely divided apical cell, whose every segment immediately builds up a branch primordium at ¼ divergence; the axis bears 4 pericentral cells which produce externally one or two layers of accessory cells of the same length, and then small-celled, parenchmatous cortex; every 6th branch-primordium develops into a long shoot and others into tufts of 2-3 mm long, uncorticated, dwarf shoots; the dwarf shoots produce small, thin, stalked, club-shaped brachyblasts that are probably propagule-like bodies; reproductive organs unknown.

Recently, a second species, *M. afaqhusainii* Shameel, was identified from the coast of Pakistan (Shameel, 1999). The occurrence of all known populations of *Melanothamnus* on the coasts of Arabian Sea (40° to 70° E and 01° to 25° N) might be significant from a biogeographical point of view. The present studies on the populations of Pakistan and the existing literature reveal that the plants of *Melanothamnus*, present in the Arabian Sea can be divided into three groups *viz.*, (1) Somalian populations, (2) Yemeni and Omani populations and (3) Pakistani populations. These groups resemble one another in several characters but they also differ in a few important characters.

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These differences are so prominent that they appear to form separate taxonomic entities. In this study morphological characters, anatomical features and reproductive structures of *M. afaqhusainii* from the coast of Pakistan, have been investigated in detail and compared with the populations of *M. somalensis* from Somalia, Yemen and Oman.

Materials and Methods

Three populations of *M. afaqhusainii* Shameel were found growing at Buleji, Paradise Point and Cape Monze, west of Karachi (Pakistan). The plants become visible in September, attain a maximum density during October-November covering the lower littoral rocks like a carpet and start degenerating in December. Each population was examined from September to December 1985-1995. The external characters were studied in freshly collected specimens and cellular structures were examined in material fixed in 4% formalin-seawater solution. The sectioning and staining were carried out as described earlier (Afaq-Husain & Shameel, 1996). Selected specimens were mounted on herbarium sheets and are kept in the herbarium of PCSIR, Karachi (CLH) and Seaweed Herbarium, MAH Qadri Biological Research Centre, University of Karachi (KUH-SW).

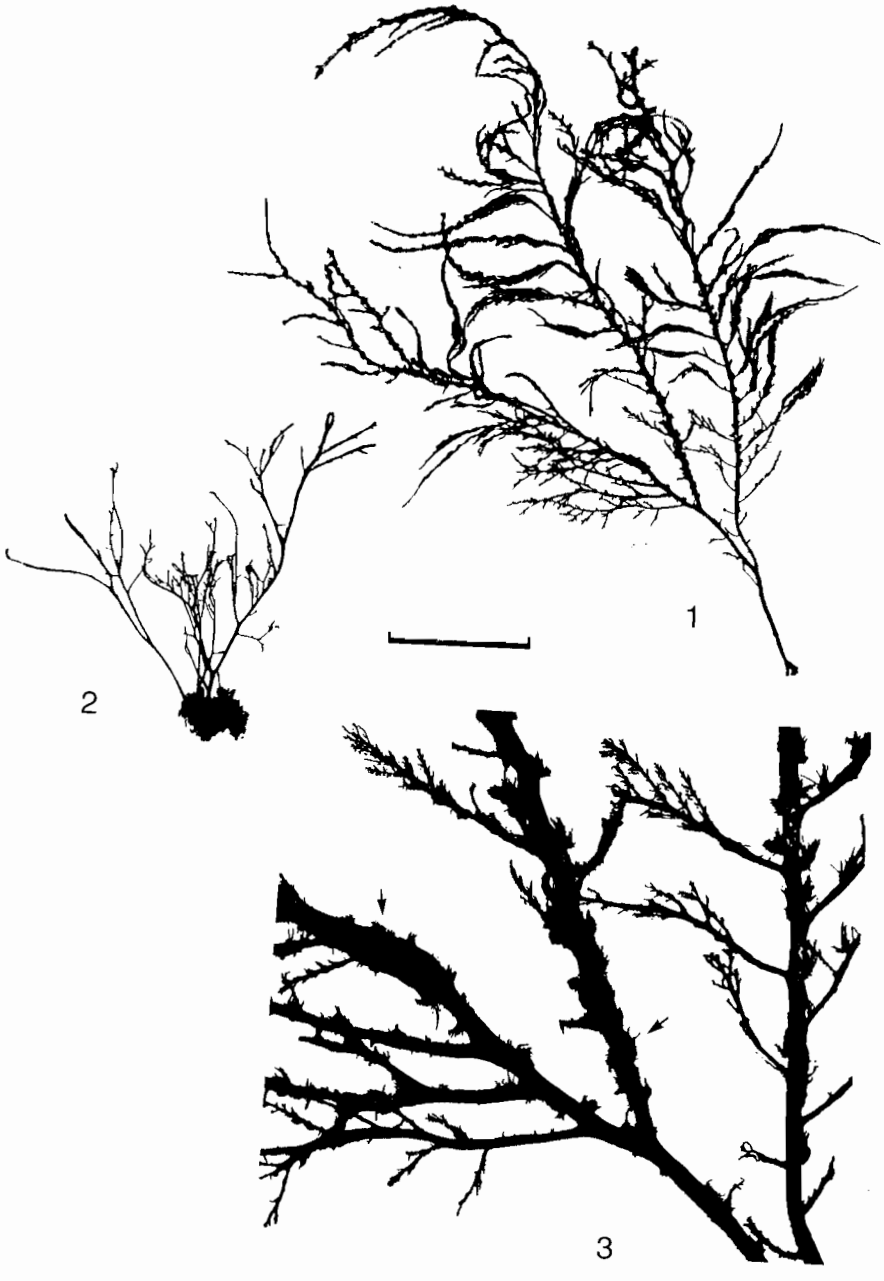
Specimens examined: Buleji (*leg.* S. Afaq-Husain 17-10-85, 12-11-85, 7-10-86, 27-9-88, 16-9-89, 9-991; *leg.* M. Shameel 10-10-90, 27-10-91, 13-11-93, 9-10-95); Paradise Point (*leg.* S. Afaq-Husain 23-12-88; M. Shameel 9-11-91, 14-10-94, 21-11-95); Cape Monze (*leg.* S. Afaq-Husain 16-10-85, 6-10-86; M. Shameel 7-11-92, 27-9-95).

Results

Melanothamnus afaqhusainii has been described by Shameel (1999) providing certain taxonomic features. Following is the result of a detailed investigation of its morphological characters, anatomical features and reproductive structures.

External characters: The plants are up to 80 cm long, much branched, reddish brown, tough to the touch and firmly attached to rocks. Shedding of plants takes place from above the holdfast and their remnants are seen attached to rocks for longer period. At low tide the plants are seen lying on rocks, densely covering the surface or hanging from rock-margins in the form of tufts of cords. The attachment system is primarily a small disc-like holdfast, which gradually increases in size by elongation of its cells in the form of small (microscopic) rhizoidal projections. The discs of neighbouring plants fuse together forming a large compound holdfast, which also produces new, upright shoots. A holdfast may become as broad as 5 cm or more and bear many plants (Figs. 1 & 2).

The main axis (primary axis) is terete or cylindrical, up to 4 mm in diam., bearing small to large branches irregularly and radially at varying distances. The large branches, in turn, behave similarly up to 4th (-5th) order. Their width decreases with the increase in the order of branching. The individual branches appear of uniform thickness from the base for more than half of their length and in the distal region they taper to



Figs. 1-3. *Melanothamnus afaqhusainii* Shameel: 1. Plant showing habit. 2. Several plants arising from a compound holdfast. 3. A part of the specimen enlarged to show small branches with distichous and dentate appearance and tufts of propagule producing branches (arrow).
(Scale: Figs. 1, 2 = 60 mm, Fig. 3 = 15 mm).

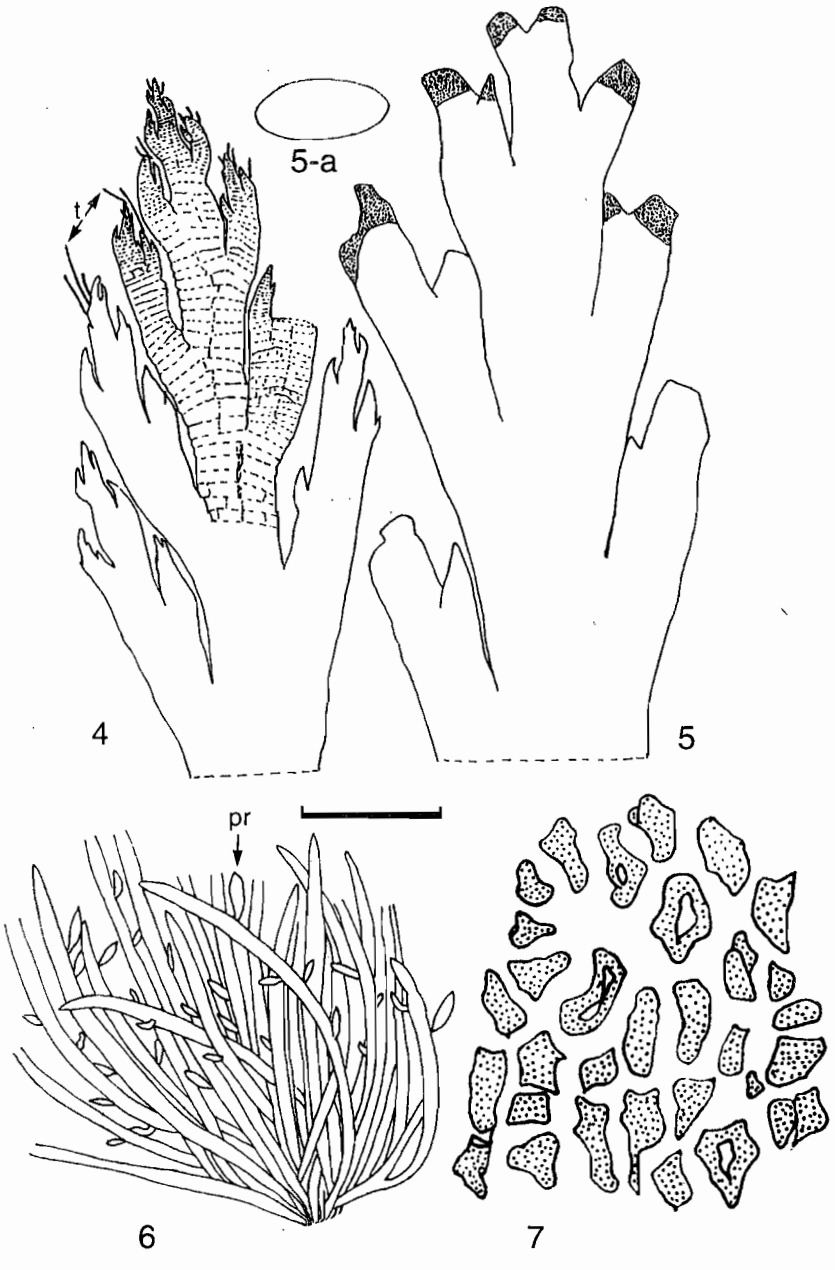
broadly conical apices. The small branches, which may be terete to compressed, are further branched in such a manner that they appear distichous and dentate to the naked eye (Figs. 3-5). They are present over the entire thallus so that their dentation may be the 3rd to 7th order of branching. The thallus is particularly characterized by the presence of small tufts of special, minute, filiform "dwarf branches", which resemble the filaments of *Polysiphonia* in appearance and produce propagule-like bodies. These tufts are visible with the naked eye and are so close together that they give a zig-zag appearance to the mother axis (Figs. 1, 3 & 6).

Surface view of the thallus: Tips of branches under the microscope are found to bear repeated branchlets, closely arranged in a distichous manner (Figs. 4 & 5). Each branchlet bears a dome shaped apical cell, which is 7-20 μm long x 10-20 μm broad at the base. Below it 2-3 (-4) cells are observed clearly in the form of thin, oblique segments or discs, placed one above the other. Further to the proximal side these segments become larger and each produces 4 pericentral cells, which in turn get covered by daughter cells of the same length. At this stage the pericentral cells cannot be observed, but the accessory cells are still seen arranged in definite tiers (Fig. 8). The older tiers are 33-72 μm long (high), and their cells are 9-33 μm broad. Further away the tiers are not visible as they are covered externally by a cortex of smaller cells. The cells are thick walled and highly variable in size and shape (Fig. 7). The colourless walls are 6-14 μm thick, and the coloured part of the cell is 19-105 μm long x 9-36 μm broad. The cells, as small as 12 x 12 μm, are also seen at distances all over the thallus surface.

The discoid cells, proximal to the apical cell, are also observed to bear a daughter cell which is broad and directed outwardly upward and may be distinguished as the initial of the future branch. In a given plane of a branch-tip it is observed on every fourth tier or sometimes on two consecutive segments with a ¼ divergence. Proximal to the discoid cells, few-celled daughter branchlets are seen arising in the same position with their apical cells resembling the above-mentioned initials. Further downwards, the shoot apex is observed to bear daughter branches distichously, one daughter branch arising from every 6th or 7th tier of the shoot (Fig. 8). The daughter branches repeat the same cellular construction during their own development.

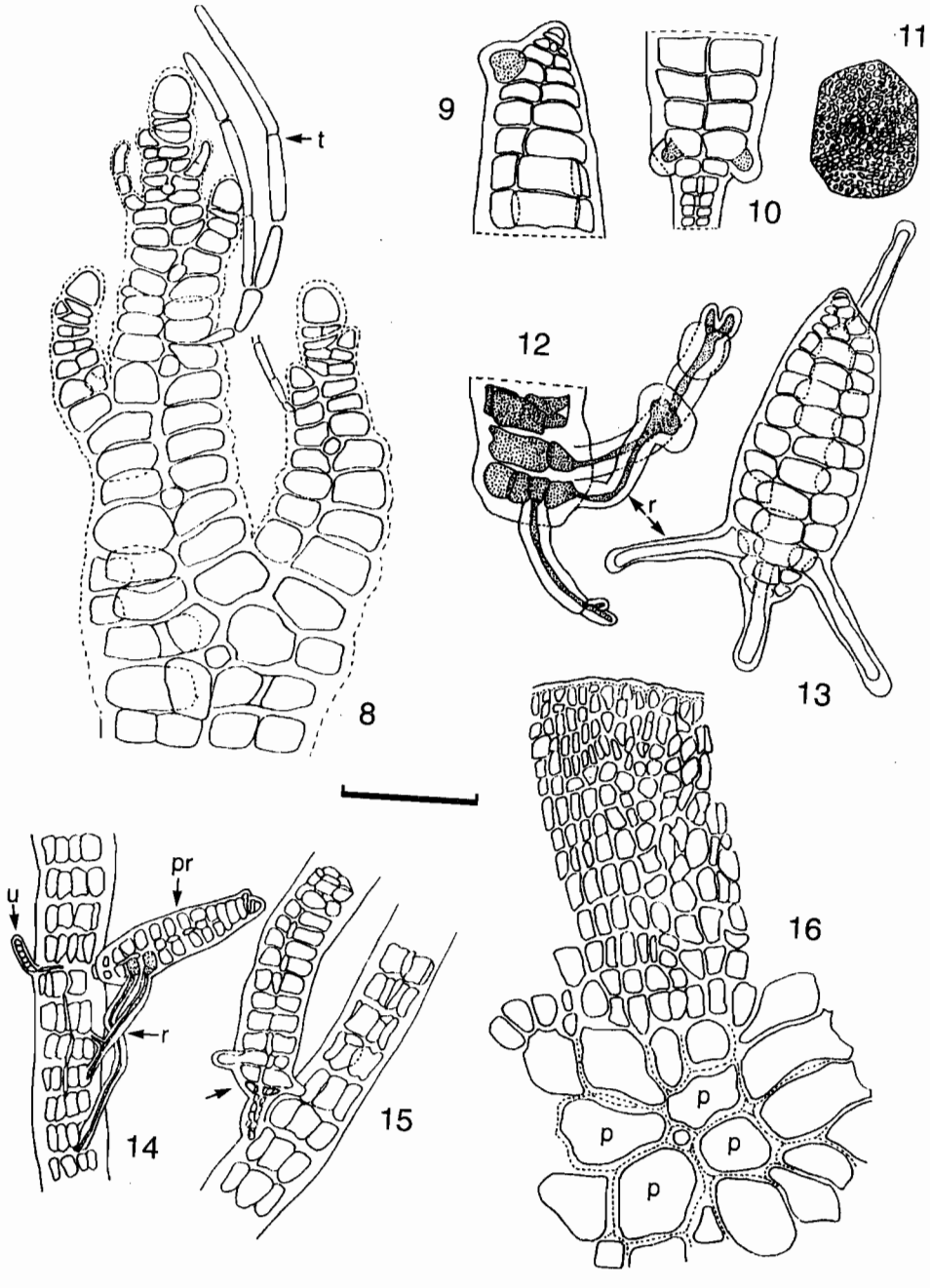
The distal tiers also bear trichoblasts which are pigmented, branched, up to 300 μm long and 8 μm broad. They are deciduous and not found on older tips. Their initials are easily distinguishable from the branch initials by their narrow and elongated shape (Figs. 4 & 8). In addition to the above, there are microscopic, few celled, dormant branchlets which are found irregularly on the entire thallus. They remain small and develop into other type of branches, especially dwarf branches (propagule-producing branches) as and when needed. In the older branch system up to 250 μm long distal part of the tips become dark in colour due to some secretion, and they appear dormant. Their apices are usually observed broken (Fig. 5).

Dwarf branches: They appear to be special branches which are borne in small tufts on entire thallus (Figs. 1 & 3). The tufts consist of few to many fine thread-like structures or filaments, which resemble the fronds of *Polysiphonia* in appearance. These filaments



Figs. 4-7. *Melanothamnus afaqhusainii*: 4 & 5. Outline of tips of branches as seen in low power showing distichous arrangement of daughter branches. 4. Tips of terete and active branches. 5. Tips of compressed and dormant branches with dark coloured apical parts (dotted). 5-a. Outline of T. S. of a compressed branch. 6. Tuft of propagule-producing branches (dwarf branches) in low power. 7. Surface view of thallus. (pr = propagule, t = trichoblast.

(Scale: Figs. 4, 5, 5-a = 450 μm, Fig. 6 = 1000 μm, Fig. 7 = 100 μm).



Figs. 8-16. *Melanothamnus afaqhusainii*: 8. Surface view of an active tip of a branch in high power showing cellular details. 9. Distal part of a propagule bearing a branch initial like cell (dotted). 10. Proximal part of a propagule bearing one rhizoidal initial (doted) on each side of basal tier. 11. A cell from middle tier of a mature propagule in high power showing its contents. 12. Proximal part of a propagule bearing 3 rhizoidal filaments (2 are branched and one is pad like at the tips). 13. A mixture of propagule which gave rise to 4

rhizoidal branches (one distal 3 proximal) prior to shedding from mother plant. 14. A dwarf branch (propagule-producing branch) in low power bearing one young, uniseriate branchlet and a propagule which has given rise to 2 rhizoidal branches which have penetrated the mother filament (dwarf branch). The propagule appears due to the formation of a branch initial cell. 15. A dwarf branch bearing a daughter branch whose basal part resembles the basal part of a propagule (arrow). 16. A part of T. S. of primary axis showing cellular details. (p = pericentral cells, pr = propagule, r = rhizoidal branches, t = trichoblast, u = uniseriate branchlet.

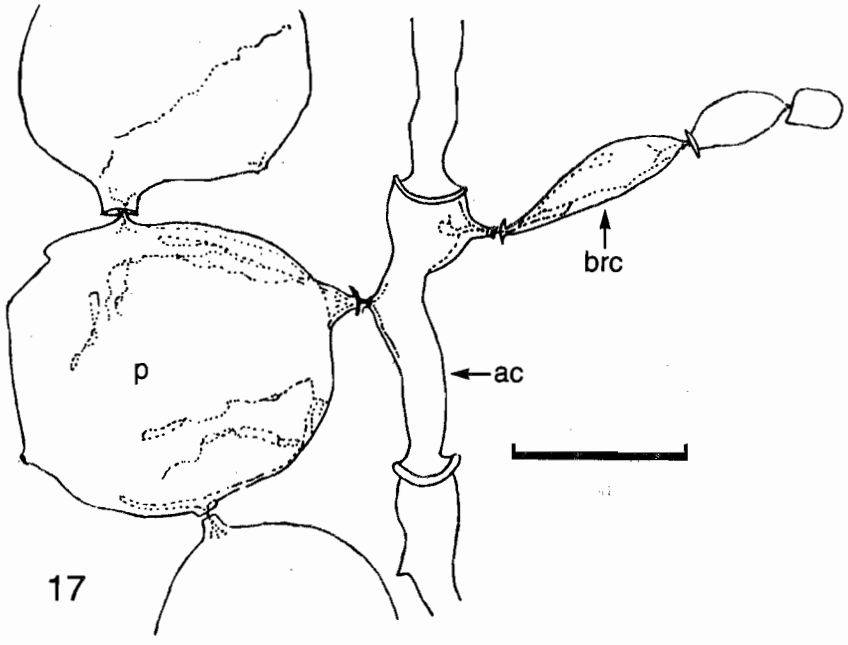
(Scale: Figs. 8, 11 = 50 μm, Figs. 9, 10, 12, 13 = 100 μm, Figs. 14, 15 = 200 μm. 16 = 450 μm).

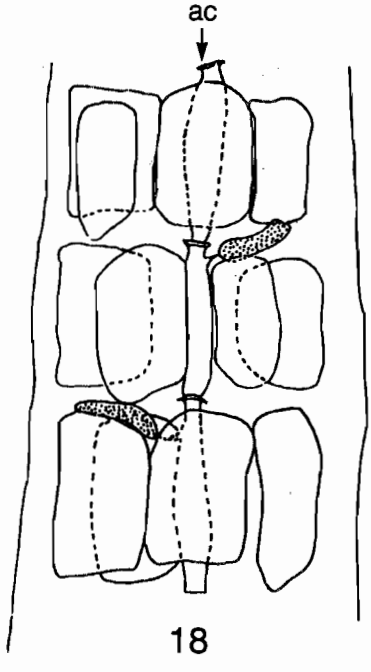
are up to 3.5 mm long and 90-130 μm broad in the middle, narrow at the base, becoming conical at the apex, usually unbranched but branched ones are also found. Primarily they are made up of 4 pericentral cells which soon divide longitudinally or obliquely in such a manner that they appear to have more than 4 pericentrals (Figs. 6, 14 & 16). The pericentral cells are 50-80 μm long and 40-50 (-55) μm broad. These dwarf branches form propagule-like bodies, which are borne irregularly.

Anatomical features: The anatomical studies of mature thalli reveal that the cells are arranged in two definite zones. The inner zone consists of large colourless cells, 3-4 layers in thickness, and the outer zone of small coloured cells, many layers in thickness. In primary axis the outer zone is found to consist of up to 14 layers of cells. The transverse section (T. S.) of frond (Fig. 16) shows a small axial cell in the centre, 30-100 μm broad, surrounded by 4 pericentrals, up to 350 μm broad, which in turn are surrounded by 2-3 layers of similar cells, up to 650 μm broad. In T. S. these cells appear irregular in shape and size, but they are more or less equal in length (length along the long axis of the thallus) to the respective axial cell. The longest cell teased out from this region was 1070 μm long.

External to the above, the cells become immediately small, and pigmentation starts in them. In T. S. the cells appear rectangular to squarish, rounded to elongated, arranged in radial rows usually with their long axis at right angle to the thallus surface, 80-150 μm x 30-75 μm broad. The epidermal cells also appear longer than broad (in T. S.), 40-86 (-137) μm long x 26-50 (-72) μm broad. Sometimes they remain smaller in length than breadth and appear to be arranged with their long axis parallel to the thallus surface. The size and arrangement of epidermal cells is markedly uneven. They are covered with a thick cuticle, which is up to 28 μm thick and undulated in outline. All the cells are thick-walled.

Microscopic examination of cytoplasmic connections shows that the pericentral cells originate from the middle of the axial cell and branch initials from its distal end (Figs. 17 & 18). The neighbouring cells (either of same or different tiers) become attached to each other through secondary cytoplasmic connections. The branch cells make their way through the joining place of lower and upper tiers as uniseriate filaments. They produce pericentrals after their emergence on the surface of the thallus. The daughter filaments, destined to give rise to propagule-producing branches, divide repeatedly into many branch filaments within the cortical region of the mother thallus and each, on emergence on surface, further divide into several filaments so that a tuft of filaments is formed (Fig. 6).





Figs. 17-18. *Melanothamnus afaqhusainii*: 17. Teased out cells of primary axis after treatment with HCl + aniline blue showing attachment of pericentral cells and branch cells to axial cell. 18. Part of a dwarf branch (slightly tapped under coverslip) showing shape and arrangement of pericentral cells, branch cells (dotted) and axial cells. (ac = axial cell, brc = branch cell, p = pericentral cell.
(Scale: 50 μm).

Reproductive structures: No reproductive bodies except propagule-like structures are observed in these plants. These structures are found in large numbers on special branches and are most probably meant for vegetative reproduction. These are more or less spindle-shaped, broader in the middle, becoming conical at the distal end with a clear dome-shaped apical cell (Figs. 6 & 13). Some appear bifid due to the formation of a branch initial which develops in such a manner that it gives a bifid appearance to the body (Figs 9 & 14). They are (200-) 240-300 (-365) μm long and (60-) 70-90 (-100) μm broad in the middle and consist of 13-18 (-21) tiers of cells including the apical cell. Each tier except the apical or subapical ones, bears 4 pericentrals and no cortication is seen outer to them. In surface view the cells, in the middle of the body, appear smaller in length (along the long axis of the body) than their breadth and are 24-41 μm long x 30-50 μm broad. Each propagule-like body bears a stalk proximally measuring up to 60 μm long x 36 μm broad and consisting of 3-4 very reduced tiers of cells, the latter being 7-15 μm long or broad. In one case the stalk was found 70 μm long with 6 tiers of cells.

On maturation the cells of the presumed propagule are filled with very small, colourless, refractive bodies, which may be reserve food material (Fig. 11). The cells of proximal and distal region are observed developing into rhizoid-like structures either before or after shedding of propagules (Figs 10 & 13). The rhizoidal filaments are up to 26 μm broad and may turn into pad-like structures at their distal ends or divide into 2-3 small finger-like branches (Fig. 12). The latter are found penetrating the host axis on which the propagule might rest after shedding (Fig. 14). Up to 4 rhizoidal structures are seen arising at a time from a single propagule-like body before or after shedding (Fig. 13).

Discussion

Melanothamnus remained a poorly known monotypic genus as described from Somalia with the type species *M. somalensis* (Falkenberg, 1901). The present populations under study agree with most of the generic characters. Since this genus was described to be monotypic, these characters can also be ascribed to its type species *M. somalensis*, and hence to the Somalian plants. The populations from Pakistan differ from those of Somalia in height as well as in the shape of propagule-like bodies. The latter (brachyblasts) are club-shaped in Somalian plants but they are clearly conical (spindle like) in the plants of Pakistan (Figs. 13 & 14).

Wynne & Banaimoon (1990) have also described *M. somalensis* from Yemen and Oman. The differences between plants of Pakistan and Yemen are greater than those between Pakistan and Somalia. The plants from Yemen are much smaller (up to 30 cm high, similar to those of Somalia which are up to 36 cm high), and their axes bear a narrower, outer, small-celled cortical zone as compared to the plants from Pakistan. In T.S. of axes the outer zone is few celled in the plants from Yemen (Fig. 10, p. 217; Wynne & Banaimoon, 1990), but it is 7-14 celled thick in the plants from Pakistan (Fig. 16). The shape of propagule-like bodies is similar in plants from Yemen and Pakistan but their position appears to be entirely different. In the populations from Pakistan the propagule-like bodies are borne on special dwarf branches (Figs. 6 & 14

similar to Somalian plants) which were not described by Wynne & Banaimoon (1990) in their plants. They rather said that propagule-like bodies "are produced in abundance in the distal portion of the plants", their fig. 9 (p. 217) shows that these bodies are attached to common branches.

In the present studies the dwarf branches were observed to be borne in tufts on the entire thallus, which in turn bear propagule-like bodies. Some difference is apparent in size and number of tiers of cell in the propagule-like bodies. The latter are 200-210 μm long x 68-84 μm broad and consist of 13-15 tiers of cells in Yemeni plants but these are found up to 365 μm long x 100 μm broad, consisting of up to 21 tiers of cells in Pakistani populations. Furthermore, the plants were described to occur in wave exposed situations (upper part of littoral zone which remains wet with splashing water waves) on the Oman coast, whereas these plants grow in the mid-littoral zone on the coast of Pakistan. Wynne & Banaimoon (1990) have suggested: "It appears possible that these apparent propagules in *Melanothamnus* are an adaptation to their extremely exposed, upper littoral habitat". But in Pakistan these populations do not grow in extremely exposed, upper littoral habitat. The three populations also differ in the position of long shoots or branches. The latter emerge from every 5th segment in Yemeni plants (Wynne & Banaimoon 1990), from every 6th segment in Somalian plants (Kylin, 1956) and from every 6th or 7th segment in Pakistani plants as found in the present studies.

The germination of propagule-like bodies has not been studied. However, many shed or attached propagule-like bodies have been observed with rhizoidal filaments which help the propagule to remain firmly attached to the substratum, probably till the next season. The cells of propagule-like bodies contain reserve food material which probably helps the propagule to remain alive till the next season, then develop into a new plant. Some branch filaments were observed with their base similar to that of propagule-like bodies (Fig. 15). This probably indicates that sometimes the apical cell of propagule-like bodies becomes active and cuts off daughter segments proximally which behave in a normal way, giving rise to a branch filament, when still attached to the mother filament through its stalk.

These observations support the idea of Wynne & Banaimoon (1990) that such structures serve as a means of external reproduction in these plants. Moreover, such structures resemble the asexual reproductive propagules of *Polysiphonia propagulifera* (Womersley, 1979). It is probable that on maturation these bodies (propagules) fall on the substratum, where they become firmly attached through their rhizoidal filaments which are produced for this purpose before or after their shedding. They remain dormant till favourable conditions arrive in the next season when their apical cell becomes active and start producing daughter segment proximally which behaves normally and develops into new plants.

The results of the present studies would suggest that the populations growing in Somalia, Yemen/Oman and Pakistan differ from each other in important characters (Table 1). These differences are enough to treat each group into separate taxonomic entity. The plants from Yemen and Oman were not available for examination and therefore *M. somalensis* is left in its original position for further investigations. The populations from Pakistan are quite different and are, therefore, treated as a different specific entity as *M. afaqhusainii*.

Table 1. Comparative features of different populations of *Melanothamnus*.

Characters	*M. somalensis*		*M. afaqhusainii*
Specimens from	Somalia	Yemen/Oman	Pakistan
Maximum height	up to 36 cm	up to 30 cm	up to 80 cm
Shape of axis	Cylindrical	Cylindrical	Cylindrical
Branching	Radial	Radial	Radial
Apical part of branch	Distichous	----	Young branches compressed and distichous
Emergence of branches	From every 6th segment	From every 5th segment	From every 6th/7th segment
Dwarf branches	Present in tufts	----	Present in tufts
Nature of dwarf shoots	Uncorticated, 2-3 mm long	----	Uncorticated but pericentral cells apparently become thicker, up to 3.5 mm long
Propagule-like bodies	Club-shaped borne in dwarf shoots	Conical/spindle shaped, borne on ordinary branches	Conical/spindle shaped, borne on dwarf shoots
Cortex	Parenchymatous, thin walled	Cells appear thick walled	All cells are thick walled
Small celled outer cortical zone in axis	----	Few cell-layered, broad	7-14 cell-layered, broad
Length of propagule-like bodies	----	200-210 μm	(200-) 240-300 (-365) μm
No. of tiers of cells in propagule	----	13-15	13-18 (-21)

--- No available information (see e.g., Kylin, 1956; Wynne & Bonaimoon, 1990).

In a recent study (Hayee-Memon & Shameel, 1999), *M. afaqhusainii* was found to contain certain interesting sterols *viz*., ostreasterol, 7-oxocholesterol and Somalenone (a rare C26 sterol), cholesterol was present in greater proportion (74.36%). Oleic acid was found in high quantity (27.88%) in this alga followed by margaric acid (21.82%), while palmitic acid was present in small amount (7.7%) contrary to other investigated red seaweeds of Karachi (Shameel, 1990). Therefore, *M. afaqhusainii* appears to be an interesting object for further phycochemical studies.

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