

## The red algal genus *Reticulocaulis* from the Arabian Sea, including *R. obpyriformis* sp. nov., with comments on the family Naccariaceae

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*Reticulocaulis obpyriformis* Schils, sp. nov. is described from the south coast of Socotra Island (Yemen), and a second species, *R. mucosissimus*, is recorded from a similar upwelling area in the Arabian Sea (Masirah Island, Oman). These are the first published records of Naccariaceae for the Indian Ocean and end the monospecific, Hawaiian-endemic status of *Reticulocaulis*. Features distinguishing *R. obpyriformis* from *R. mucosissimus* include its more sparsely branched thallus, obpyriform rather than cylindrical inner cortical cells, the presence of short moniliform laterals of small spherical cells on the cortical filaments, monoecious rather than dioecious gametophytes, and the direct development of spermatangia from catenate mother cells. The morphology and anatomy of the gametophytes of this heteromorphic genus are discussed in relation to those of other naccariacean genera.

### INTRODUCTION

Recent phycological studies in the Arabian Sea and the northern Indian Ocean have resulted in the description of new taxa (Wynne 1999a) and a plethora of new records (Wynne & Banaimoon 1990; Wynne & Jupp 1998; Wynne 1999b, 2000) indicative of a unique marine benthic flora. The south-west monsoon that results in upwelling along the south-eastern coastline of the Arabian Peninsula (Currie *et al.* 1973; Ormond & Banaimoon 1994) is an important physical phenomenon influencing these neritic ecosystems and their biotas, particularly those of Masirah Island (Oman) and the Socotra Archipelago (Yemen), which support a seasonally rich diversity of gelatinous red algae (Schils & Coppejans 2002). Among the more unexpected of the algae recently discovered, there are two species of *Reticulocaulis*, a hitherto monotypic genus thought to be confined to Hawaii in the central Pacific Ocean and a member of the relatively little-known and infrequently encountered family Naccariaceae.

Following the recommendations of Kylin (1928), Svedelius (1933) and Feldmann & Feldmann (1942), the Naccariaceae is generally included in the order Bonnemaisoniales, based on details of gonimoblast development and the presence of nutritive-cell clusters on the carpogonial branch (Chihara & Yoshizaki 1972), an ordinal placement supported by ultrastructural characters of the pit plugs (Pueschel & Cole 1982). Womersley (1996), however, commented that the family might not be related to the Bonnemaisoniaceae, because of some seemingly major differences in the carposporophyte, such as a diffuse rather than compact gonimoblast and the complete absence of a pericarp. Abbott (1999) recently placed the Naccariaceae in the Gigartinales without specifying her reasons for the transfer. The Naccariaceae currently comprises the genera *Atractophora* P. Crouan & H. Crouan, *Naccaria* Endlicher and *Reticulocaulis* I.A. Abbott. Despite consisting of only seven species, of which five belong to *Naccaria*, the

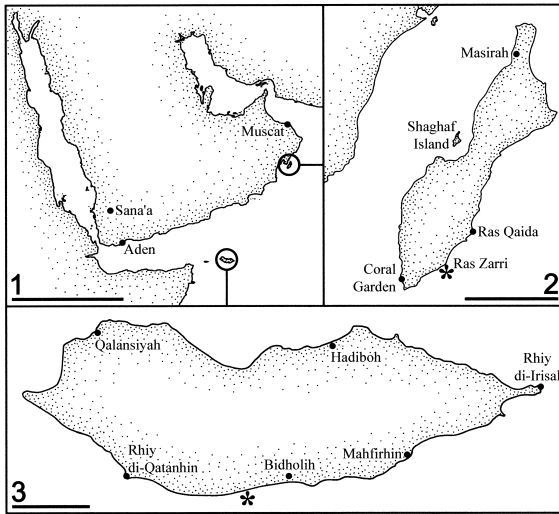
family is widely distributed throughout the Atlantic and Pacific Oceans. Although a single robust female gametophyte of *N. naccarioides* (J. Agardh) Womersley & I.A. Abbott is known from the Indian Ocean coast of Western Australia (GEN-10793e, MELU: *leg.* G.T. Kraft & G.W. Saunders, 7 October 1995, Pinaroo, Western Australia, 32.20°S, 115.45°E), the present article is the first published report on a member of the Naccariaceae from anywhere in the Indian Ocean.

### MATERIAL AND METHODS

The east coast of Masirah Island (Oman; 20.42°N, 58.79°E; Figs 1, 2) and the south coast of Socotra (Yemen; 12.47°N, 53.87°E; Figs 1, 3) are influenced by a seasonal coastal upwelling from May to September, during the south-west monsoon. The specimens of this report were found in similar habitats around Masirah and Socotra, viz. rocky platforms at 10–20 m depth on which macroalgae were the most abundant benthic organisms, interspersed with isolated small hard and soft coral colonies. Gelatinous red algae such as *Dudresnaya* P. Crouan & H. Crouan, *Gibsmithia* Doty, *Platoma* Schousboe *ex* Schmitz and *Predaea* De Toni species (Schils & Coppejans 2002), were particularly conspicuous during the early-winter and late-spring periods, other associated algae being *Amphiroa* J.V. Lamouroux spp., *Callophycus* Trevisan sp., *Caulerpa peltata* J.V. Lamouroux, *Euptilota fergusonii* Cotton, *Galaxaura marginata* (Ellis & Solander) J.V. Lamouroux, *Halimeda* J.V. Lamouroux spp., *Lobophora variegata* (J.V. Lamouroux) Womersley *ex* Oliveira, *Rhodymenia* Greville spp., *Spatoglossum asperum* J. Agardh, and *Udotea indica* A. Gepp & E. Gepp.

Specimens of *Reticulocaulis* were collected by the first author during field trips to Masirah Island on 2–30 November 1999 and Socotra on 26 March–7 May 2000, the subtidal habitats being accessed by means of SCUBA. The collected algae were pressed on herbarium sheets, with portions preserved in a 5% formalin-seawater solution. Herbarium sheets, wet spec-

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**Figs 1–3.** Collection sites of *Reticulocaulis* in the Arabian Sea. Scale bars = 1000 km (Fig. 1); 20 km (Figs 2, 3).

**Fig. 1.** The Arabian Peninsula showing Masirah Island and Socotra. **Fig. 2.** Sample site 9 (asterisk; 20.199°N, 58.715°E), near Ras Zarri, off Masirah Island, Oman.

**Fig. 3.** Sample site ALG-40 (asterisk; 12.303°N, 53.843°E), west of Bidholih, off Socotra, Yemen.

imens and microscope slides are deposited in GENT (Ghent University Herbarium, Krijgslaan 281/S8, 9000 Ghent, Belgium). Slides and formalin-preserved samples of Hawaiian *R. mucosissimus* I.A. Abbott were kindly supplied by I. A. Abbott of the Bernice Bishop Museum. Herbarium sheets of *N. corymbosa* J. Agardh and *N. wiggii* (Turner) Endlicher were borrowed from the National Herbarium of the Netherlands (L). Material for microscopical examination was stained with aniline blue, fast green or Lugol's Iodine (for rhodoplasts). Material for nuclear and pit-connection studies was stained using Wittmann's aceto-iron-haematoxylin-chloral hydrate (Wittmann 1965), following the procedures of Hommersand & Fredericq (1988). Anatomical and reproductive characteristics were observed from tissue squashes (whole-mounts in a 50% corn syrup-water solution, containing a few drops of phenol) using light microscopy (Leitz Diaplan). Photographs were taken with a Wild MPS51 35 mm camera and on an Olympus DP50 digital camera.

## RESULTS

### *Reticulocaulis mucosissimus* I.A. Abbott 1985, p. 555

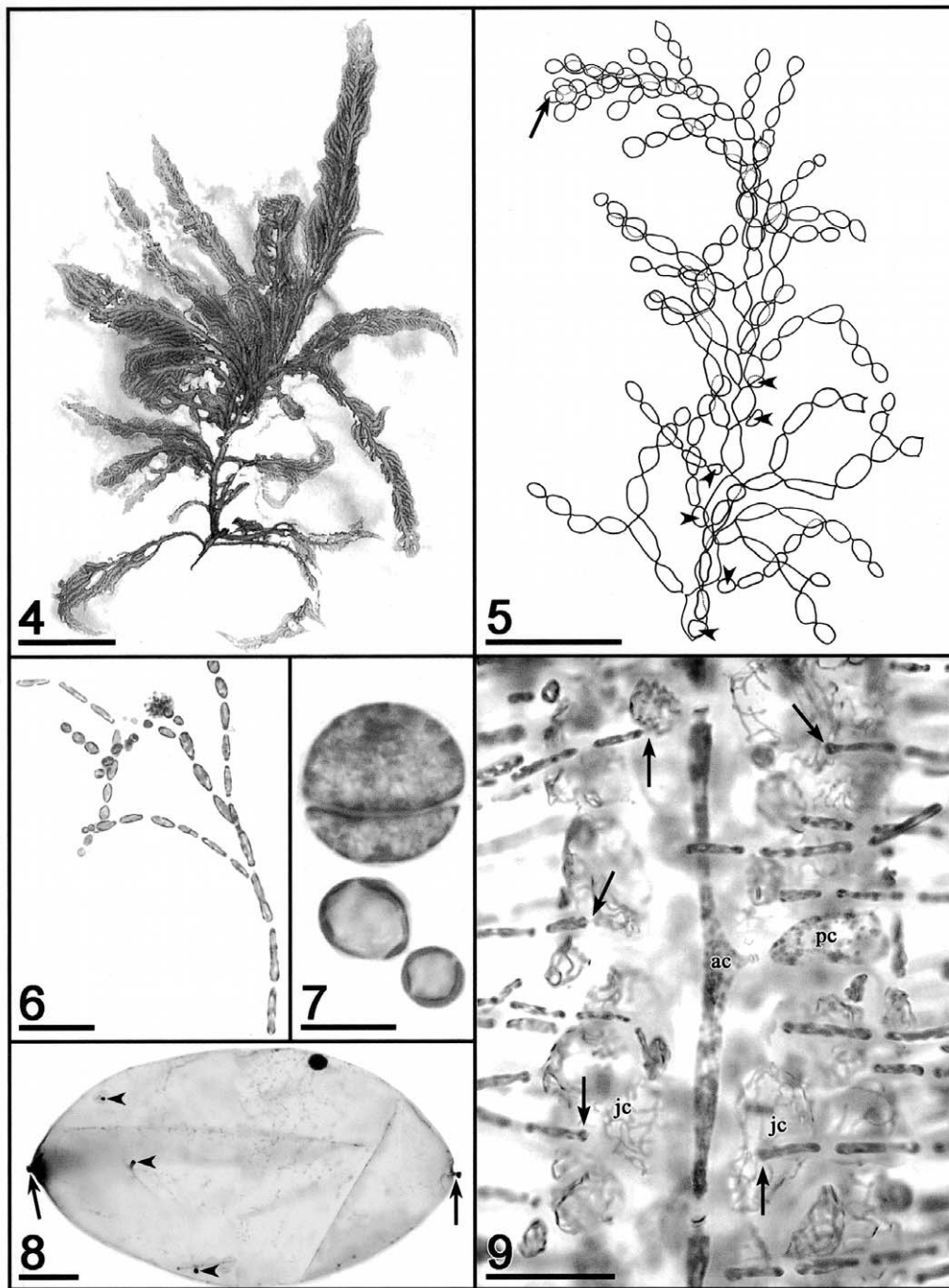
**SPECIMENS EXAMINED:** **Oman.** Masirah Island (Figs 1, 2): sample site 9 (20.199°N, 58.715°E), close to Ras Zarri. A rocky platform at 9 m depth with scattered rocky outcrops in an area of strong surge (Schils, 9 November 1999). MAS 138: female (Fig. 4) and male gametophytes. **Hawaii.** Mahukona, north-west coast of Hawaii. Plants growing on dead coral at a depth of 9 m (K. J. McDermid, 26 May 1998). Formalin sample IA 23471 (female gametophyte) and slide KM 4481 (female gametophyte); Kawaiolo, Oahu Island (W. H. Magruder & S. Carper, 10 May 1985). Slide IA 17225: female gametophyte.

Thalli are bright red, mucilaginous and attached by a discoid holdfast (Fig. 4). Omani plants reach 13 cm in length and grow from dome-shaped apical cells that divide obliquely, the immediate daughter cells being aligned in a nearly straight row (Fig. 5). The axial cells are slender and elongate, those lying 1 mm away from the apical cells having length-width ratios of  $> 4:1$ . The first periaxial cell (the 'superior' periaxial) is cut off three axial cells from the apex, and superior periaxial cells on successive segments are produced in an irregular  $\frac{1}{4}$  spiral. A second periaxial cell (the 'inferior' periaxial) is always positioned proximal to the first. It is generally cut off in cells positioned 15–20 cells away from the apex (Fig. 5) and at a  $90^\circ$  angle to the first periaxial cell. At the same time, several rhizoidal outgrowths develop from both periaxial cells; these outgrowths branch. Besides differing in the timing of their initiation, the shapes of the two periaxial cells are also dissimilar: the superior periaxial cell becomes elongated and rectilinear, whereas the inferior one remains spherical (Fig. 5). The inferior lateral becomes the more developed of the two laterals and occasionally gives rise to indeterminate branches as it continues growing and initiates periaxial cells. Infrequently, an axial cell can initiate a third periaxial cell, which develops like the superior lateral. The derivatives of the periaxial cells (from about the 15th axial cell) differentiate rapidly by branching and cell elongation into determinate filaments that constitute the cortex. The inner cortical cells are cylindrical (Fig. 6), whereas the outer cells remain ovoid to (sub)spherical.

Two-celled propagules, reaching  $16.5 \mu\text{m}$  in diameter (Fig. 7) and developing terminally on many of the cortical filaments, were observed on slide IA 17225 of a specimen from Hawaii. One or two axial cells below the site where the second periaxial cell first forms, both periaxial cells initiate rhizoidal downgrowths. The periaxial cells and the rhizoidal downgrowths inflate into what were termed 'jacket cells' by Abbott (1985), viz. cells that mutually cross-connect by lateral secondary pit connections (Fig. 8) and constitute a sheath around the central-axial strand (Fig. 9). While maturing, the pit connections of the jacket cells attenuate and become difficult to distinguish, which results in a seemingly parenchymatous covering. Before the covering is complete, the jacket cells initiate secondary cortical filaments that are either fasciculate or unbranched, as well as secondary rhizoidal downgrowths. In older parts of the thallus, the jacket cells become densely covered by these secondary rhizoidal filaments, which rarely branch and form uniseriate rows that cross one another, but actually constitute a single layer.

The rhodoplasts are discoid but like erythrocytes in shape ( $2\text{--}4 \mu\text{m}$  in diameter), having centres that are thinner than the margins.

Female gametophytes have carpogonial branches that are of accessory origin; they were found throughout the thallus in various stages of development. Near the apex, carpogonial branches arise singly from either of the periaxial cells. Further down the thallus, they also develop from other jacket cells (rhizoidal filament cells) and the lower cortical filament cells. Pairs of carpogonial branches on a single supporting cell are infrequently seen. The branches consist of 7–13 equally staining cells, which, following the terminology of Lindstrom (1984), can be designated by numbers starting with the carpogonium (#1). Eccentric positioning of the primary pit con-



**Figs 4–9.** *Reticulocaulis mucosissimus*. ac, axial cell; jc, jacket cell; pc, periaxial cell. Vegetative features. Scale bars = 2 cm (Fig. 4); 50  $\mu$ m (Figs 5, 6); 10  $\mu$ m (Fig. 7); 50  $\mu$ m (Figs 8, 9).

**Fig. 4.** Female gametophyte (pressed herbarium specimen) from Masirah Island; MAS 138.

**Fig. 5.** Apex of an indeterminate axis, showing the apical cell (arrow) and periaxial cells (arrowheads); KM 4481.

**Fig. 6.** The transition from cylindrical inner- to spheroidal outer-cortical cells; MAS 138.

**Fig. 7.** Two-celled propagules on a Hawaiian specimen; IA 17225.

**Fig. 8.** Detail of an inflated jacket cell in a Hawaiian specimen, showing primary (arrows) and lateral secondary pit connections (arrowheads); IA 23471.

**Fig. 9.** Sheath of jacket cells around the central-axial strand, showing secondary cortical filament initiation (arrows); MAS 138.

nections results in a zigzag arrangement of carpogonial branch cells when viewed dorsally or ventrally (Fig. 12). The carpogonial branch curves sharply towards the axis bearing it and the carpogonium arises adaxially on cell #2, the hypogynous cell (Figs 10, 11). The initially short and reflexed trichogyne can elongate to over 500  $\mu\text{m}$  (Figs 12, 13; Abbott 1985).

Cell #2 initiates a cluster of 4–6 branched filaments of tightly packed nutritive cells (Fig. 14), whereas cells #3 and #4 tend to bear a primary, slightly branched lateral, a second slightly more branched lateral and 1–3 small clusters of ramified nutritive cells (Fig. 13). Primary laterals, 6–16 cells in length and branched to two orders, form adaxially on most of the remaining carpogonial branch cells, the longest occurring on the most proximal cells (Figs 13, 14). Any of the cells proximal to cell #4 may ultimately bear either an abaxial or an adaxial second sterile filament.

Upon presumed fertilization, the carpogonial branch cells and the basal cells of the sterile laterals inflate, and both the pit connections and the nuclei of these cells enlarge substantially (Fig. 15). The gonimoblast initial develops directly from the fertilized carpogonium (Fig. 16); at the same time, the nutritive cells fuse directly with the hypogynous cell through their pit connections, which retain their original size or expand only slightly as the pit plugs break down (Fig. 17). The passageways that are now open between the hypogynous cell and the nutritive-cell clusters presumably become paths for direct nutrient transport to the developing gonimoblast. The carposporophyte remains compact, does not intermingle with vegetative tissue, and lacks a pericarp. Ovoid carposporangia ( $40 \times 30 \mu\text{m}$ ) terminate the branches of the compact gonimoblast (Fig. 18); cystocarps at various stages of development are found scattered within the cortex and reach 330  $\mu\text{m}$  in diameter.

Spermatangia are produced in terminal dendroid clusters on separate male gametophytes, the fertile axes often being accompanied by a sterile sibling cortical filament of one or two cells (Fig. 19). Spermatangial mother cells initiate 1–3 spermatangia (Fig. 20).

Tetrasporangial thalli were not collected in the course of this study and are unrecorded for the genus. In line with findings for other genera of the Naccariaceae (Jones & Smith 1970; Boillot & L'Hardy-Halos 1975), *Reticulocaulis* is presumed to have a heteromorphic life history involving a diminutive system of prostrate filaments bearing terminal tetrahedral tetrasporangia. Growth of Hawaiian *R. mucosissimus* in culture, reported by Abbott (1999, p. 123), resulted in a microscopic filamentous phase but no production of tetrasporangia.

#### *Reticulocaulis obpyriformis* Schils, sp. nov.

Affinis *R. mucosissimis* Abbott (1985) sed differt characteribus pluribus. Gametophyta monoica; thallus pallido-roseolus pallidus, usque ad 15 cm altus, rami indeterminatis laxe et irregulatim ramificantibus. Cellulae corticis obpyriformes cylindricae; rami breves cellulis parvis sphaericis in filamento corticato, rarus evolutantes in axes indeterminatos; interdum trichomata in cellulis terminalibus rel subterminalibus corticis portata; cellulae axiales intra 1 mm sub apice latae ad 70(–80)  $\mu\text{m}$ . Spermatangia evoluta e filamentis corticalis cellulis distalibus. Praesentia duorum ramorum carpogonialis in cellula basali frequentior quam in *R. mucosissimo*. Filamenta lateralia secunda persaepe in cellulis proximis ramorum carpogonialis.

Similar to *R. mucosissimus* Abbott (1985) but with the following distinguishing characters: gametophytes monoecious; thalli pale pink, to 15 cm high; branching of indeterminate axes loose and irregular. Cortical cells obpyriform and cylindrical; cortical filaments bearing short laterals consisting of small spherical cells and potentially developing into indeterminate axes; hairs occasional on terminal and subterminal cortical cells; axial cells broadening to 70(–80)  $\mu\text{m}$  within 1 mm of the apices. Spermatangia developing directly from catenate series of distal cortical cells. Supporting cells bearing two carpogonial branches occur more frequently than in *R. mucosissimus*. Secondary laterals common on proximal carpogonial branch cells.

HOLOTYPE: GENT, SMM 446 (Fig. 21)

TYPE LOCALITY: West of Bidholih, south coast of Socotra Island (Figs 1, 3). Sample site ALG-40 (12.303°N, 53.843°E): a rocky platform at –19 m covered with thin layers of sand and punctuated by deeper sand patches (Schils, 30 April 2000).

ETYMOLOGY: *obpyriformis*, refers to the inverse pear shape of the cortical cells.

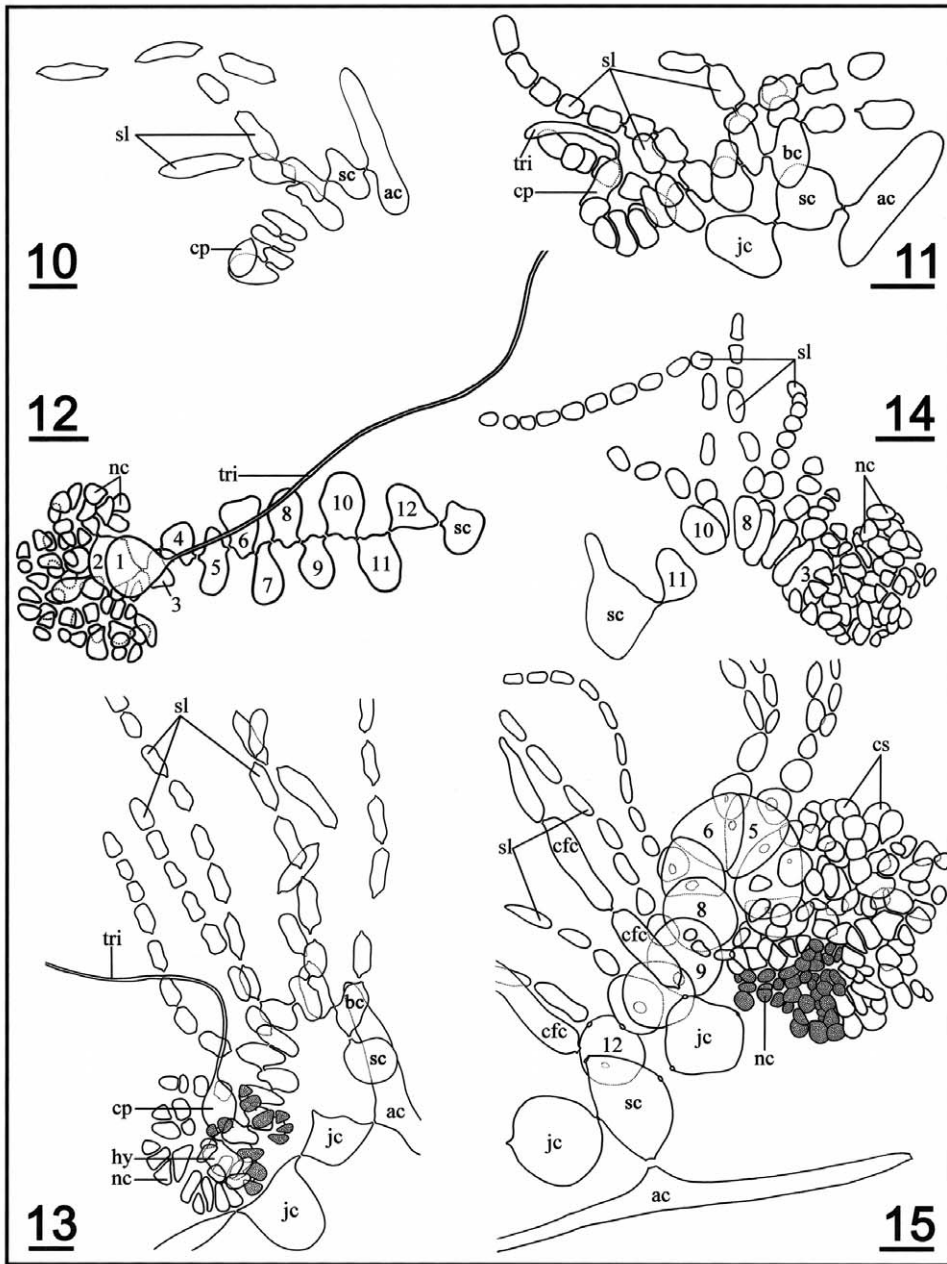
The thalli are terete, pale pink, and up to 15 cm in length (Fig. 21). Branching is irregularly radial, with a sparse development of up to four orders of indeterminate laterals. The dome-shaped apical cell divides obliquely, the immediate derivatives forming a sinusoidal pattern before the axial cells become aligned (Fig. 23).

Within 1 mm of the apices, the axial cells broaden to attain length–width ratios of  $< 4:1$  (Fig. 22). The superior periaxial cell is cut off at about the third axial cell behind the apex, the ‘phyllotaxy’ on successive segments being alternate (Fig. 23). Inferior periaxial cells, rhizoidal downgrowths and laterals develop from about the 40th axial cell downwards, at which time the phyllotaxy of the determinate laterals tends to become an irregular  $\frac{1}{4}$  spiral, because the inferior periaxials set in at a 90° angle to the superior periaxial cells. Derivatives of the inferior periaxial cells become more strongly developed than those of the superior cells and initiate the occasional indeterminate branch when the cortical filament continues growing and initiates periaxial cells. Third-order periaxial cells are very infrequently initiated in older parts of the thallus; they develop cortical filaments and jacket cells like the other periaxial cells.

The lower cells of the cortical filaments are predominantly obpyriform (Figs 22, 24), although cylindrical to barrel-shaped cells also occur (Fig. 24). The sizes and contours of the cortical cells change rather abruptly distally, from being elongated, obpyriform or cylindrical, and up to 90  $\mu\text{m}$  long by 27  $\mu\text{m}$  wide, to being small, spherical and 4–6  $\mu\text{m}$  in diameter. Hairs develop occasionally on terminal and subterminal cortical cells (Fig. 25), but propagules were not observed.

Certain cortical filaments bear short moniliform laterals of small spherical to ovoid cells (Fig. 24); these laterals can bear spermatangia, less often carpogonial branches, or may transform directly into indeterminate axes (the atypical way of indeterminate lateral formation: Fig. 23).

Several orders of rhizoidal downgrowths develop from the periaxial cells, the cells becoming inflated and linked by lateral secondary pit connections (Fig. 26) and forming a sheath around the axial strand (Fig. 27), in which the pit connections attenuate and become obscure. These jacket cells are spheroidal and may give rise to secondary cortical filaments. In older parts of the thallus, the jacket cells become densely covered



**Figs 10–15.** *Reticuloaulis mucosissimus*. Carpogonial and carposporophyte morphology (MAS 138). ac, axial cell; bc, basal cell of carpogonial branch; cfc, cortical filament cells; cp, carpogonium; cs, carposporangium; jc, jacket cell; nc, nutritive-cell cluster; sc, supporting cell of carpogonial branch; sl, sterile lateral; tri, trichogyne. Scale bars = 10  $\mu$ m.

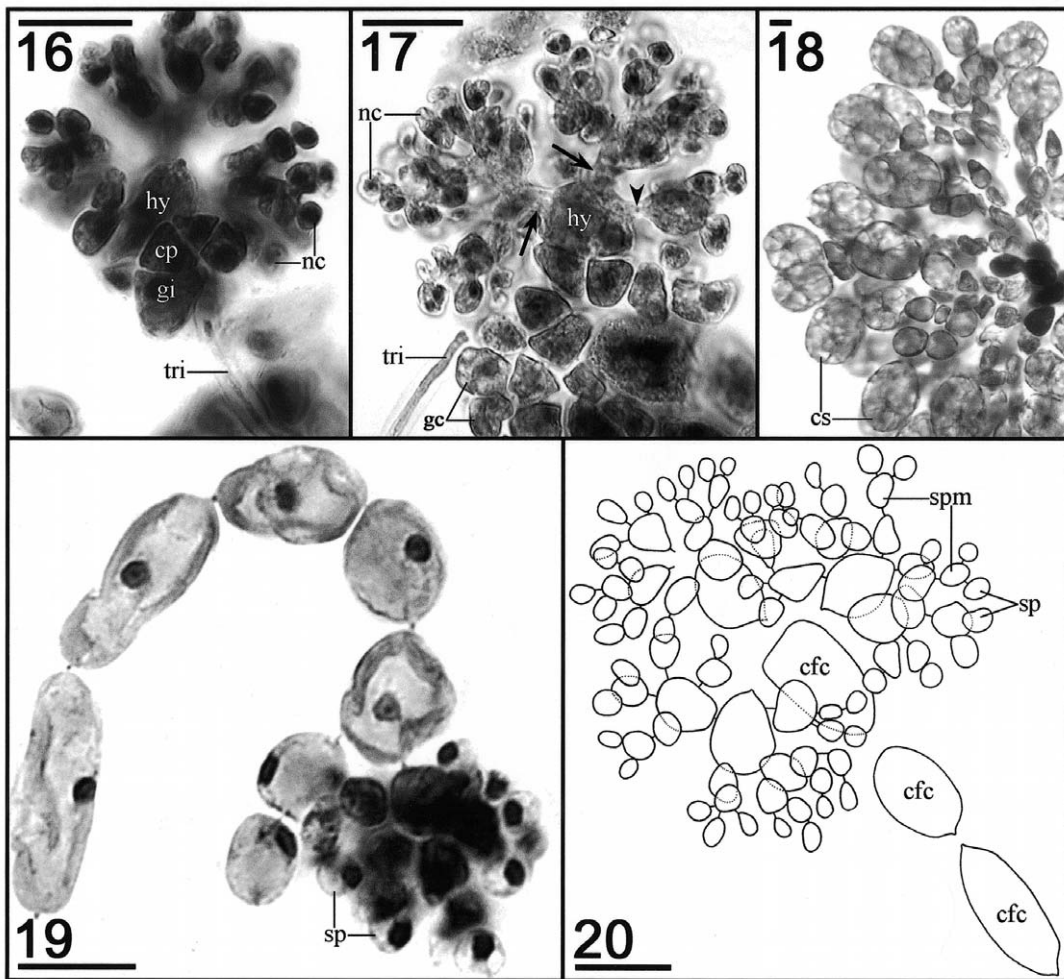
**Fig. 10.** Seven-celled carpogonial branch before elongation of trichogyne from the carpogonium, with sterile laterals growing from the lower cells.

**Fig. 11.** Young carpogonial branch, on which the carpogonium has produced a reflexed trichogyne and sterile laterals have arisen from most of the proximal cells. A jacket cell has also been initiated by the supporting cell.

**Fig. 12.** Dorsal view of a mature carpogonial branch, showing the zigzag arrangement of the cells and densely clustered nutritive cells borne on the hypogenous cell (cell #2).

**Figs 13, 14.** Lateral views of carpogonial branches bearing nutritive-cell clusters on the hypogenous cell and on cell #3, cell #4 (shaded) and lengthy sterile laterals on more proximal cells.

**Fig. 15.** Early carposporophyte development, showing the nutritive-cell clusters (shaded) and carposporangium initiation. The carpogonial branch cells and the basal cells of the sterile laterals inflate and pit connections widen. Cortical filaments arise from jacket cells.



**Figs 16–20.** *Reticulocaulis mucosissimus*. Cystocarpic and spermatangial features. (MAS 138). cfc, cortical filament cell; cp, carposporangium; cs, carposporangium; gc, gonimoblast cell; gi, gonimoblast initial; hy, hypogynous cell; nc, nutritive cell; sp, spermatangium; spm, spermatangial mother cell; tri, trichogyne. Scale bars = 10  $\mu$ m.

**Fig. 16.** Division of the (presumably fertilized) carposporangium to produce the gonimoblast initial. Nutritive-cell filaments are borne on the hypogynous cell, and the trichogyne is still attached to the carposporangium.

**Fig. 17.** Fusion of the nutritive-cell clusters with the hypogynous cell through primary pit connections (arrowhead), in which the pit plugs progressively break down (arrows), resulting in broad open passageways. Gonimoblast cells are larger and more angular than nutritive cells and abut the clusters next to the remnant trichogyne.

**Fig. 18.** Ovoid terminal carposporangia borne on angular penultimate cells of branched gonimoblast filaments.

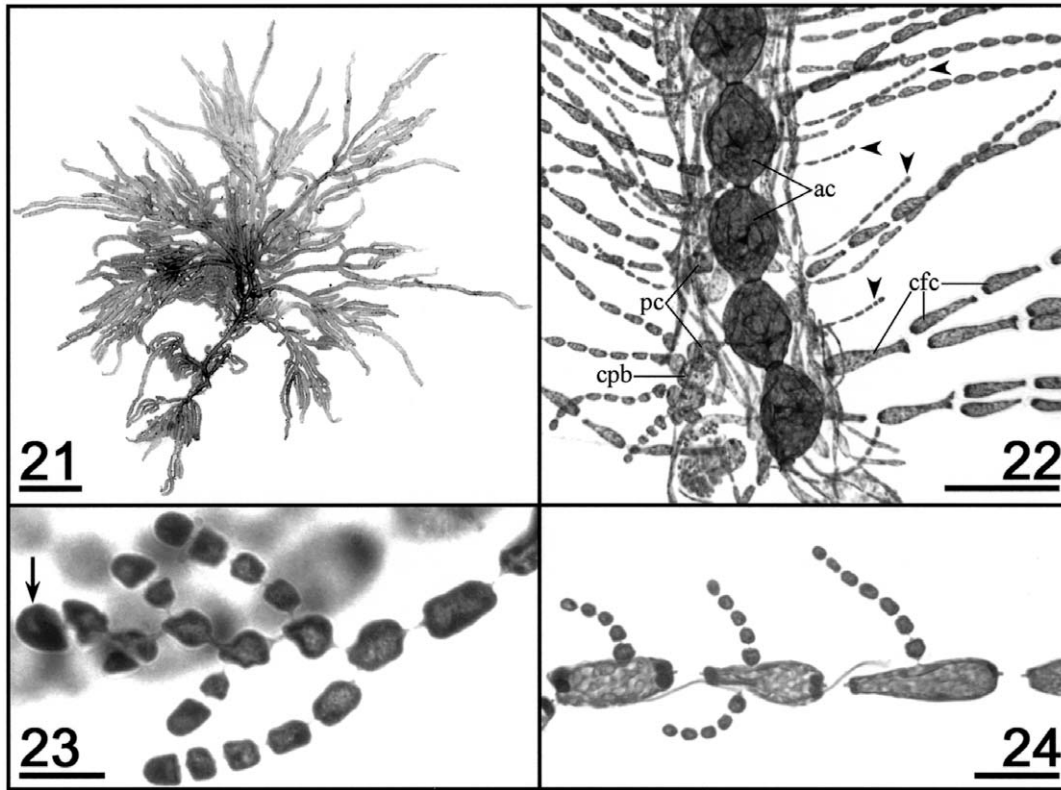
**Fig. 19.** Spermatangia forming in dendroid clusters on one of a pair of ultimate branches of a cortical filament, the cells of the second branch remaining sterile.

**Fig. 20.** Detail of a dendroid spermatangial cluster: the spermatangia are borne mostly in pairs on subterminal mother cells.

by rhizoidal filaments. The rhizoidal filaments develop from periaxial cells and other jacket cells; they branch (Fig. 28), and some initiate secondary cortical filaments (Figs 22, 27, 28).

The rhodoplasts are discoid, have a distinctive 'erythrocyte' appearance (Fig. 29), and are 2–4  $\mu$ m in diameter. As in *R. mucosissimus*, the rhizoidal and jacket cells contain fewer rhodoplasts than do the cortical cells, and older axial cells virtually lack them altogether.

The gametophytes are monoecious. Spermatangia develop on terminal (Fig. 25) and subterminal cortical cells, with up to nine fertile axial cells forming in series (Fig. 30). Unlike in *R. mucosissimus*, the spermatangia tend to be borne directly on fertile axial cells rather than on terminal mother cells of dendroid cortical filaments. Carposporangial branches are scattered throughout the thallus in various states of development. The carposporangial branch is 7–13 cells long, the supporting cell being one of the periaxial cells, a jacket cell (rhizoidal fila-



**Figs 21–24.** *Reticulocaulis obpyriformis*. Habit and vegetative features (SMM 446). ac, axial cell; cfc, cortical filament cell; cpb, carpogonial branch; pc, periaxial cell. Scale bars = 2 cm (Fig. 21); 100  $\mu$ m (Fig. 22); 10  $\mu$ m (Figs 23, 24).

**Fig. 21.** Holotype (a pressed monoecious specimen).

**Fig. 22.** Bead-like, inflated axial cells jacketed by derivatives of the periaxial cells and by rhizoids that give rise to unbranched secondary cortical filaments (arrowheads). Primary cortical filaments of obpyriform cells and a carpogonial branch are borne on the periaxial cells.

**Fig. 23.** Direct transformation of a short moniliform branch of a cortical filament into an indeterminate lateral, as indicated by the sinusoidal development of the axis behind the apical cell (arrow) and the alternate production of periaxial cells and cortical filaments.

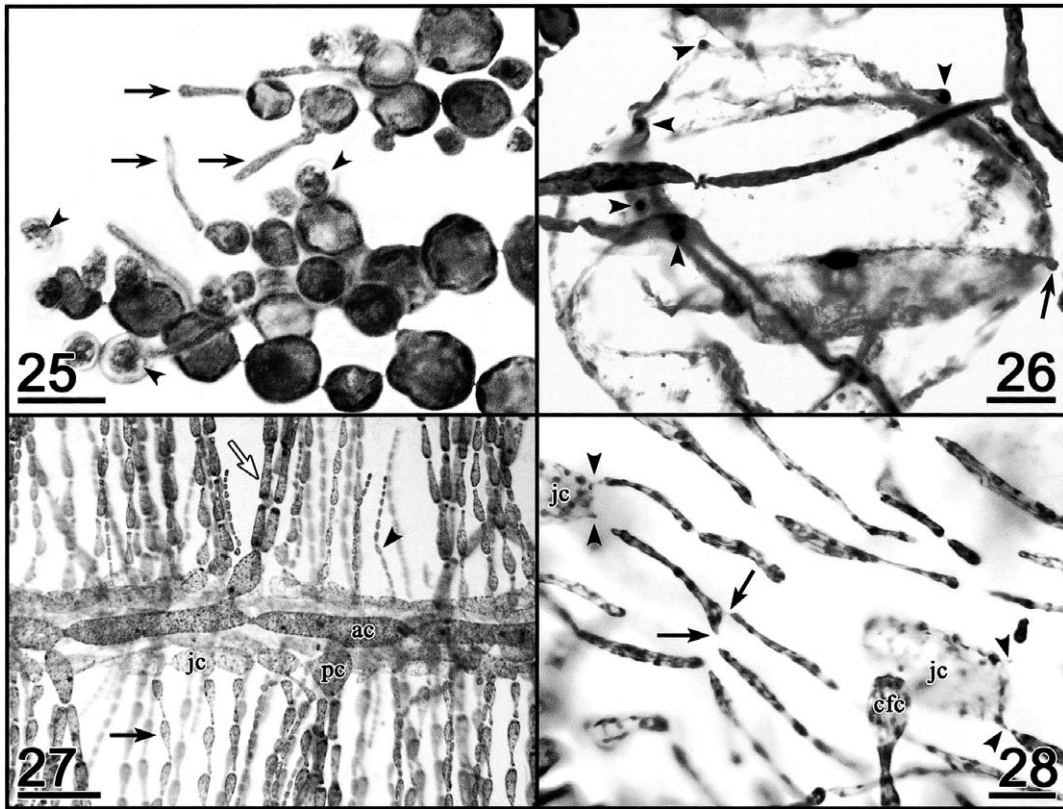
**Fig. 24.** Obpyriform and cylindrical cortical cells bearing single or paired moniliform laterals of restricted growth.

ment cell) or a lower cortical filament cell. The presence of two carpogonial branches on a single supporting cell occurs more frequently than in *R. mucosissimus* (Fig. 31). The hypogynous cell produces 4–6 branched clusters of densely aggregated nutritive cells. Cells #3 and #4 generally each bear two longer branched laterals and 1–3 small nutritive-cell clusters. The carpogonial branch cells proximal to cell #4 bear a long primary sterile lateral and may ultimately come to bear an abaxial or adaxial second sterile lateral. As the carpogonial branch matures, sterile laterals become progressively more branched. Upon fertilization, the carpogonial branch cells and the basal cells of the sterile laterals inflate, both the pit connections and nuclei of these cells enlarging substantially. The gonimoblast initial develops directly from the fertilized carpogonium. The nutritive cells did not stain because their contents were rapidly emptied, and thickened strands between the nutritive-cell clusters and the hypogynous cell were not seen. Mature carposporophytes were not observed and hence no measurements of cystocarpic structures (diameter of cystocarps and carposporangia) could be made.

Tetrasporophytes are unknown.

## DISCUSSION

The Arabian collections of *Reticulocaulis* extend the known distribution of the Naccariaceae from the Atlantic and the Pacific to the north-western Indian Ocean. Both species occur there in habitats similar to that occupied by *R. mucosissimus* in Hawaii, the Hawaiian populations forming part of a 'spring flora', which consists mainly of gelatinous species of *Acrosymphyton* L.G. Sjöstedt, *Dudresnaya*, *Gibsmithia* and *Schmitzia* P.C. Silva growing in areas scoured by waves 4–10 m in height (I.A. Abbott, personal communication). The strong seasonality of members of the Naccariaceae has been documented previously (Dixon & Irvine 1977; Womersley 1996) and we suspect that seasonal growth in the northern Indian Ocean may be related to day-length changes and water temperature. The occurrence of *R. mucosissimus* in Hawaii and Oman corresponds to previous reports of a biogeographical affinity of certain Arabian Sea biota with distant regions in the Pacific (Coles 1995: Hawaii; Wynne 2000: Japan; Schiils & Coppejans 2002: Australia). These disjunct distributions can be explained by (1) undersampling of subtidal habitats



**Figs 25–28.** *Reticulocaulis obpyriformis*. Habit and vegetative features (SMM 446). ac, axial cell; cfc, cortical filament cell; jc, jacket cell; pc, periaxial cell. Scale bars = 10  $\mu\text{m}$  (Fig. 25); 20  $\mu\text{m}$  (Fig. 26); 100  $\mu\text{m}$  (Fig. 27); 20  $\mu\text{m}$  (Fig. 28).

**Fig. 25.** Hairs (arrows) and spermatangia (arrowheads) developing from terminal and subterminal cortical cells.

**Fig. 26.** Primary pit connection (arrow) and lateral secondary pit connections (arrowheads) of an inflated jacket cell covered by a narrow rhizoidal filament.

**Fig. 27.** Axial cells surrounded by a sheath of jacket cells, which develop branched (black arrow) and unbranched secondary cortical filaments (arrowhead). Primary cortical filaments (open arrow) are borne on the periaxial cells.

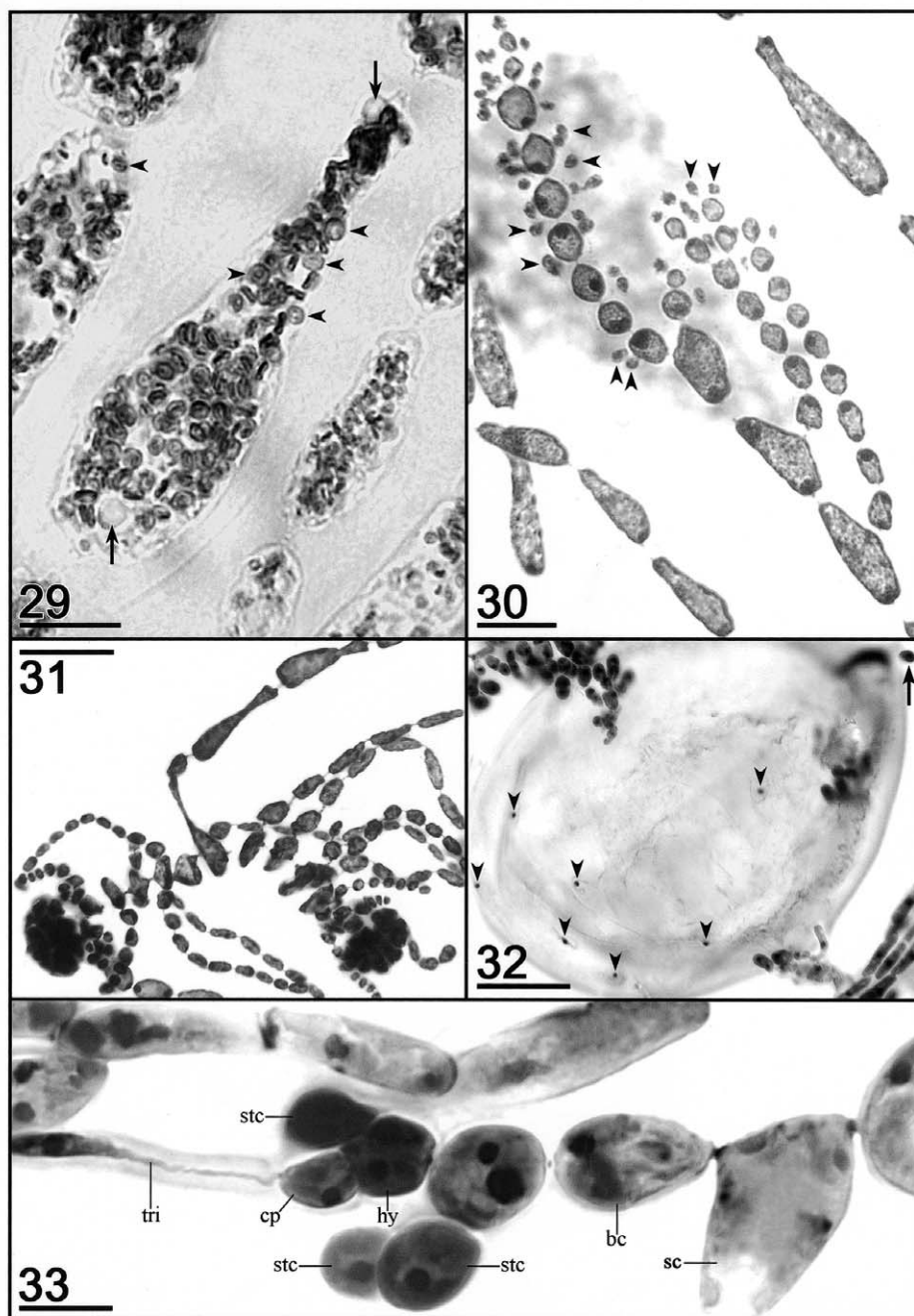
**Fig. 28.** Jacket cells that initiate multiple rhizoidal filaments (arrowheads), which branch (arrows), and secondary cortical filaments.

within the Indo-Pacific (Schils & Coppejans 2002); and (2) being relicts of Miocene distributions, which were altered as a result of changes in the current patterns (Hommersand 1986) that formerly connected these regions, separating the refugia that are subject to seasonal temperate water (Schils *et al.* 2001). However, because of the seasonal appearance of the Naccariaceae and their generally infrequent occurrence, few data are available and it is currently not possible to favour either of the two hypotheses.

The *Reticulocaulis* species, *R. mucosissimus* and *R. obpyriformis*, are easily distinguished by various anatomical and morphological features (Table 1). In erecting the genus, Abbott (1985) distinguished *Reticulocaulis* from the closely related *Naccaria* by the different developmental pattern of the 'jacket cells' (see below), the longer and more elaborately branched carpogonial branches and the compact vs diffuse carposporophyte. However, Abbott (1985) was comparing *R. mucosissimus* with *N. naccarioides* (J. Agardh) Womersley & I.A. Abbott (previously regarded as the type species of *Neoardissonia* Kylin) and *Naccaria hawaiiiana* I.A. Abbott, rather

than with the generitype, *N. wiggii* (Turner) Endlicher. This becomes an important consideration when evaluating the contrast Abbott made between the axial sheath of *Reticulocaulis* and the 'axial pseudoparenchyma' of *Naccaria*. Abbott (1985) regarded the former as resulting from the cross-connection of enlarged periaxial- and rhizoidal-cell derivatives lying parallel to the central-axial filament in *Reticulocaulis*, whereas the multilayered axial sheath in *Naccaria* originates from several successive basal cells of the cortical filaments. Examination of material of *N. wiggii* (L 0276772: leg. P. & H. Huvé, 13 May 1963, Calanque de Sormiou, Marseilles, France; Fig. 32) and *N. corymbosa* (L 0276776: leg. A. J. Bernatowicz, 16 March 1953, Gunners Bay, east end of St David's Island, Bermuda) shows that both have similar secondary pit connections between axial-strand cells and that these become attenuate and obscure while maturing, as in *Reticulocaulis*. The sheath of jacket cells around the central axes of *N. wiggii* and *N. hawaiiiana* is composed of inflated periaxial, rhizoidal and inner cortical cells (Womersley & Abbott 1968; Boillot & L'Hardy-Halos 1975, figs 8, 13). Millar (1990) notes that the degree





**Figs 29–33.** *Reticulocaulis obpyriformis* and *Naccaria wiggii*. bc, basal cell of carpogonial branch; cp, carpogonium; hy, hypogynous cell; sc, supporting cell of carpogonial branch; stc, sterile cell; tri, trichogyne. Scale bars = 10  $\mu\text{m}$  (Fig. 29); 20  $\mu\text{m}$  (Fig. 30); 50  $\mu\text{m}$  (Figs 31, 32); 10  $\mu\text{m}$  (Fig. 33).

**Figs 29–31.** *Reticulocaulis obpyriformis*, SMM 446.

**Fig. 29.** Discoid rhodoplasts (arrowheads) with thickened rims that give them an appearance similar to that of erythrocytes; the plastids densely fill an inner cortical cell and there are also surrounding reserve vacuoles (arrows).

**Fig. 30.** Spermatangia (arrowheads) developing on terminal and intercalary cortical mother cells.

**Fig. 31.** Two carpogonial branches borne on a single supporting cell of a cortical lateral.

**Figs 32, 33.** *Naccaria wiggii*, L 0276772.

**Fig. 32.** Primary pit connection (arrow) and lateral secondary pit connections (arrowheads) on a jacket cell.

**Fig. 33.** A four-celled carpogonial branch, on which sterile cells arise from cells #2 and #3 but which lacks nutritive-cell clusters.

**Table 1.** Comparison of morphological and anatomical features in *Reticulocaulis mucosissimus* and *R. obpyriformis*.

<i>R. mucosissimus</i>	<i>R. obpyriformis</i>
dark pink	pale pink
thallus reaching 13 cm	thallus reaching 15 cm
densely branched; thallus contour tapers pyramidally at the apices because of the organisation of the short laterals	sparsely branched thallus; even the small indeterminate laterals do not branch densely
rather straight apices	sinusoidal apices
branching an irregular 1/4 spiral	branching initially alternate, later (from second periaxial cell formation onwards) an irregular 1/4 spiral
early (15–20th axial cell) appearance of second periaxial cell	late (> 40th axial cell) appearance of second periaxial cell
angular to globose jacket cells	spherical jacket cells
gradual acropetal transition of cortical cells from cylindrical to spherical; short moniliform branches of cortical filaments absent; terminal hairs lacking	abrupt acropetal transition of cortical cells from cylindrical or obpyriform to small and spherical or ovoid; short moniliform branches of cortical filaments present; terminal or subterminal hairs occasional
dioecious	monoecious
secondary laterals or rhizoidal filaments on proximal carpogonial branch cells relatively infrequent	secondary laterals or rhizoidal filaments on proximal carpogonial branch cells common
axial cells slender	axial cells broadly inflated
two-celled propagules occasional on outer cortical cells	two-celled propagules absent

of inflation of descending-filament cells in *N. naccarioides* varies in the few recorded specimens according to where in Australia they come from, thus perhaps undermining the absolute taxonomic value of the very criterion for which *Reticulocaulis* was named.

Additional features separating *Reticulocaulis* and *Naccaria* include differences in which of the periaxial cells grows out into the dominant lateral on each axial cell: supposedly it is primarily the superior in *Naccaria* and the inferior in *Reticulocaulis*. However, this criterion may not be reliable because Millar (1990) argues that the dominance of either determinate fascicle in *Naccaria* appears to be strongly affected by age or habitat.

Other characters, however, clearly distinguish *Reticulocaulis* from *Naccaria* (Table 2). The carpogonial branches are longer (7–13 cells vs 2–8 cells) in *Reticulocaulis* and develop from the periaxial cells, the jacket cells and the lower cells of the cortical fascicles, whereas in *Naccaria* species they can arise from the periaxial cells (in *N. hawaiiiana*: Abbott 1985, fig. 11), from intercalary supporting cells at various levels in the cortex (in *N. wiggii*: specimen L 0276772), or from rhizoids (in *N. naccarioides*: Womersley 1996, p. 356). The degree to which sterile laterals arise and develop on carpogonial branch cells appears to be variable in *Naccaria* species such as *N. hawaiiiana* (Abbott 1999), *N. naccarioides* (Millar 1990; Womersley 1996) and *N. wiggii* (Hommersand & Fredericq 1990; Fig. 33), but the sterile laterals in *Naccaria* never approach the degree of development seen in *Reticulocaulis*. The production of nutritive-cell clusters on the hypogynous cell is more consistent in *Reticulocaulis* than in *Naccaria* [e.g. observations of *N. wiggii*, L 0276772; *N. corymbosa* J. Agardh, L 0276776: leg. A.J. Bernatowicz, 16 March 1953, Gunners Bay, east end of St David's Island, Bermuda, and *N. naccarioides*, Womersley & Abbott (1968)], in which their presence is variable even on single plants; at times they can be absent altogether (Fig. 33). The nutritive-cell clusters on the two cells (carpogonial branch cells #3 and #4) proximal to the hypogynous cell in *Reticulocaulis* are lacking in *Naccaria*. Nutritive-cell clusters are also more numerous and more densely packed in *Reticulocaulis* than in *Naccaria* (Abbott 1985).

Perhaps the greatest difference between *Naccaria* and *Re-*

*ticulocaulis* lies in the structure of the cystocarp, which grows diffusely among cortical filaments in *Naccaria* (Dixon & Irvine 1977; Hommersand & Fredericq 1990; Millar 1990; Womersley 1996) but remains tightly compact in *Reticulocaulis*, although post-fertilization stages, such as the fusion of the fertilized carpogonium and hypogynous cell by widening of the pit connection, are similar in both genera (Millar 1990; Womersley 1996). Formation in *Naccaria* of a fusion cell that incorporates the fertile axial cell (Hommersand & Fredericq 1990; Womersley 1996, fig. 160H), however, is not seen in *Reticulocaulis* and constitutes another major difference between the two genera. The difference in the sizes of the mature cystocarp structures of *R. mucosissimus* between those reported here (carposporangium and cystocarp diameter) and those reported in Abbott (1985, p. 557, fig. 6) is probably the result of Abbott having made measurements on immature cystocarps. The specimens from Hawaii examined in this article bore cystocarp structures covering the range reportedly found in the Omani specimens.

Spermatangial organization appears to differ between species of *Naccaria* to a degree equal to that seen between the two species of *Reticulocaulis*. In *R. mucosissimus* the male gametophytes bear dense terminal clusters, in which branched laterals terminate in spermatangial mother cells (Abbott 1985, fig. 4; Figs 19, 20), whereas in *R. obpyriformis* they develop directly on outer cortical cells, as in *N. hawaiiiana* (Abbott 1985, fig. 7).

The *R. obpyriformis* type of spermatangial arrangement is also characteristic of the recently described *Liagorothamnion mucoides* Huisman, D.L. Ballantine & M.J. Wynne (2000), an enigmatic monotypic genus that the authors provisionally put in its own tribe (the Liagorothamnieae) within the family Ceramiaceae. The authors state that the post-fertilization process in *Liagorothamnion* is 'difficult to observe' and 'open to interpretation' but that it apparently involves fusion of the fertilized carpogonium by means of connecting cells or short filaments with the supporting cell, which is located at the base of a whorl-branchlet. This process is very similar to that reported for *Atractophora* (Millar 1990), to which *Liagorothamnion* thus shows a number of striking similarities. Both genera are mucilaginous, produce four whorl-laterals per axial cell,

**Table 2.** Comparison of morphological and anatomical characteristics among the genera of the Naccariaceae (Zerlang 1889; Kylin 1928; Kylin 1956; Fan 1961; Womersley & Abbott 1968; Dixon & Irvine 1977; Abbott 1985; Hommersand & Fredericq 1990; Millar 1990; Womersley 1996; Abbott 1999; this article).

Feature	<i>Atractophora</i>	<i>Naccaria</i>	<i>Reticulocaulis</i>
Branching pattern of determinate branches	whorls of four periaxial cells per axial cell	two periaxial cells per axial cell; branching on an irregular spiral of 1/4	two periaxial cells per axial cell; branching on an irregular spiral of 1/4
Sheath of inflated cells around axial strand	absent	present	present
Secondary pit connections	absent	present between jacket cells	present between jacket cells
Adventitious and rhizoidal filaments	present	present	present
Hairs on terminal cortical filament cells	present	present or absent	present or absent
Gametophytes	monoecious	monoecious or dioecious	monoecious or dioecious
Carpogonial branch (including carpogonium)	three- to four-celled	two- to eight-celled	seven- to thirteen-celled
Laterals on carpogonial branch cells	short	short	primary laterals lengthy, secondary sterile filaments present
Nutritive cells	present	absent or restricted to the hypogynous cell	always present on the hypogynous cell, small clusters on the carpogonial branch cells #3, #4
Immediate post-fertilization events	fusion of carpogonium with supporting cell by a short process	fusion of carpogonium with hypogynous cell by widening of pit connection	fusion of carpogonium with hypogynous cell by widening of pit connection
Gonimoblast composition	diffuse gonimoblast intermixed with vegetative filaments	diffuse gonimoblast intermixed with vegetative filaments	compact gonimoblast lacking vegetative filaments
Localized swelling of the branches by mature cystocarps	present	present	absent
Position of spermatangial mother cells	?	dendroid distal clusters or in catenate series	dendroid distal clusters or in catenate series

surround their central-axial filaments with a jacket of uninflated cells, have three- to four-celled recurved carpogonial branches bearing more than two sterile groups and produce a carposporophyte that surrounds the axial strand and intermingles with vegetative filaments. *Liagorothamnion* may thus prove to have a closer alliance with the Naccariaceae than with the Ceramiaceae.

Abbott (1985) has suggested that *Reticulocaulis* 'might be looked upon as reduced from *Acrosymphyton*-like forms in terms of the carpogonial branch . . .' and hence related to the Dumontiaceae, a family then placed in the order Cryptonemiales and now a member of the Gigartinales (Saunders & Kraft 1997). The proposed affinity between *Reticulocaulis* and *Acrosymphyton* is not supported by recent evidence. According to a phylogenetic analysis of the Dumontiaceae (Tai *et al.* 2001), *Acrosymphyton* (Acrosymphytaceae; Lindstrom 1987) is sister to the Gelidiales, whereas *Bonnemaisonia* is basal to the included Gigartinales and Gelidiales. Ongoing molecular research (G.W. Saunders & C.A. Maggs, personal communication) shows that the Naccariaceae is almost certainly not monophyletic (*Naccaria* groups weakly with the Bonnemaisoniaceae and may not belong in the Bonnemaisoniales, whereas *Atractophora* receives solid support as a member of the Bonnemaisoniaceae), and it clearly does not belong in either the Nemaliales or the Gigartinales. Saunders & Kraft (1997, p. 130) suggested that DNA studies of the Naccariaceae, to establish its ordinal affinities, should be a top priority for molecular systematists, and this recommendation still holds.

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