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THE SYSTEMATICS, BIOLOGY AND DISTRIBUTION OF THE GELIDIACEAE (RHODOPHYTA) OF SOUTH AFRICA AND RELATED TAXA.

By Enrico M. Tronchin

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Supervisor: Prof. J.J. Bolton

Co-supervisor: Dr. R.J. Anderson

Doctoral Degrees Board
University of Cape Town
Private Bag Rondebosch
7701 South Africa
Tel: (021) 650-2155
Fax: (021) 650-2138
vnaidoo@bremner.uct.ac.za

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ABSTRACT

The diversity, biology and distribution of the red algal family Gelidiaceae (Rhodophyta) of South Africa (and related taxa) were investigated, being relatively poorly understood and taxonomically problematic. Prior to this study, 21 species in 6 genera were reported from South Africa. Now there are 29 species in 3 genera (though molecular data indicates that there is possibly a new, undescribed genus) of which 16 are *Gelidium* species, 5 are *Pterocladiella* species and 8 are *Ptilophora* species. The phylogenetic relationships and taxonomic status of the genera *Beckerella, Ptilophora*, *Onikusa* and *Suhria*, and various species within *Gelidium* and *Pterocladiella*, were reassessed. This was done by observing external morphology and vegetative structure, and analysing chloroplast-encoded *rbc*L and nuclear-encoded LSU gene sequence data, from which phylogenetic hypotheses were inferred and pairwise sequence divergences calculated. The species of *Onikusa* and *Suhria* were subsumed into *Gelidium* and the merger of *Beckerella* into *Ptilophora* (which had not been widely accepted) was reassessed and supported on molecular and morphological grounds. In addition, two new *Ptilophora* species, *P. coppejansii* and *P. leliaertii*, were described from South Africa and *Ptilophora helenae* was resurrected (a species placed in synonymy with *Ptilophora pectinata* from Australia). Most *Ptilophora* species were found to produce thallus surface proliferations and to carry a sponge epiphyte. The hypothesis that the production of surface proliferations (formerly diagnostic of *Ptilophora*) and the presence of sponge epiphytes were causally linked was critically evaluated by observing eighty-eight *Ptilophora* specimens. Results showed that it was unlikely that surface proliferations were induced by sponge epiphytes, but that a structural relationship between the two was likely: the sponge probably exploiting a niche habitat provided by the host. A comprehensive review of *Ptilophora* was carried out including observations of type specimens and a thorough literature review. The generitype, *Ptilophora spissa*, was placed in synonymy with *P. diversifolia* having been found to be an aberrant form of *P. diversifolia*. From an analysis of *rbc*L sequences from 56 Gelidiaceae taxa including 15 of the 21 species previously reported to occur in South Africa, two new species were described from South Africa (*Gelidium declerckianum* and *G. profundum), Pterocladiella bartlettii* was reported from South Africa for the first time, and three uncertain species were described that fit a broad morphological description of *P. caerulescens*, a species not previously reported from South Africa. Vertical and horizontal distributions of Gelidiaceae species occurring in South Africa were compiled from the literature and more than 500 herbarium and field-collected specimens.

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INTRODUCTION

The Gelidiaceae is an economically important family in the Rhodophyta utilised for the extraction of the polysaccharide agar contained within the cell walls and intercellular matrix. Established by Kützing (1843), this family includes algae which are usually cartilaginous, variously shaped, producing erect and/or prostrate axes which are branched or unbranched, terete to flattened to alate and midribbed. Colour ranges from bright red to almost black or greenish-brown. Norris (1992b) clearly defined the Gelidiaceae, discussing key characteristics, whilst Hommersand and Fredericq (1988) revised the description of the Gelidiales, the order to which the Gelidiaceae belongs. In short, the Gelidiaceae is characterised by isomorphic alternation of sexual and bi-/tetrasporic generations. Plants usually have a rhizomatous/stoloniferous holdfast giving rise to erect axes. Vegetative growth takes place by transverse division of an apical cell producing a uniaxial thallus. A subapical cell forms two periaxial cells that are distichously arranged on the subsequent axial cells. The vegetative structure consists of a pigmented cortical layer surrounding a central core of elongate cells occurring in filaments. Rhizines (thick-walled, elongate, unbranched cells sometimes described as hyaline filaments that have an extremely small lumen) develop internally from cells of third- and higher order cortical filaments and grow basipetally, descending through the cortex and/or medulla. In some species they form clusters within the inner cortex forming a veritable tissue layer or may form the major part of the medulla. Reproductive structures are located mostly terminally or subterminally in slightly expanded lateral branchlets. Tetrasporangial or bisporangial plants are the more common life history phase, with tetrasporangia being cruciately (including decussately) or tetrahedrally divided. Sexual plants are dioecious, rarely monoecious. Male sori form unpigmented patches on the frond surface consisting of masses of anticlinally elongate spermatangial initials. Cystocarps may be unilocular with several carposporangia in chains, or bilocular with carposporangia occurring singly.

The Gelidiaceae contains nine genera, i.e. *Acanthopeltis* Okamura, *Capreolia* Guiry & Womersley, *Gelidium* Lamouroux, *Onikusa* Akatsuka, *Pterocladia* J. Agardh, *Pterocladiella* Santelices & Hommersand, *Porphyroglossum* Kützing, *Ptilophora* Kützing and *Suhria* J. Agardh ex Endlicher. The Gelidiaceae is one of two families in the Gelidiales, the second being the Gelidiellaceae which contains only the genus

Gelidiella Feldmann & Hamel. Some authors (Maggs and Guiry 1987, Guiry and Womersley 1992, Womersley and Guiry 1994, Yoshida 1999) do not recognise the Gelidiellaceae, and treat *Gelidiella* as a genus in the Gelidiaceae. Fan (1961) erected the Gelidiellaceae distinguished by the absence of rhizines in the cortex and medulla, and stated that a sexual generation in the life history was unknown. Maggs and Guiry (1987) described a new species *Gelidiella calcicola* Maggs & Guiry from the British Isles and northern France, simultaneously questioning the taxonomic status of *Gelidiella*. *Gelidiella calcicola* was described as having characteristic features of *Gelidiella*, namely the production of tetrasporangial stichidia with tetraspores arranged in chevrons, but differed fundamentally in that it produced rhizines in the medulla at the attachment points and had a creeping habit that never produced erect axes. The authors also cited a publication by Sreenivasa Rao and Trivedi (1980) where the cystocarp of an unidentified species of *Gelidiella* was illustrated, stating that they found the structure to be similar to that of the cystocarps of *Gelidium*. They proposed that the Gelidiellaceae and Gelidiaceae be merged, a proposal which subsequently became quite controversial. Norris (1992a) emphasised the lack of rhizines in *Gelidiella*, redefining the family and transferred *G. calcicola* to *Gelidium*. Guiry and Womersley (1992) later documented cystocarps and spermatangia in a new species, *Gelidiella minima* Guiry & Womersley, but Santelices & Hommersand (1997) transferred *G. minima* into *Pterocladiella* based on its cystocarp structure. Ultimately, Santelices (1997a) provided convincing evidence for the maintenance of the two families. He described the production of spermatangia in the type species, *Gelidiella acerosa* (Forsskål) Feldmann & Hamel, for the first time where these structures were produced in protruding, hemispherical sori located proximally on erect axes, a characteristic which sets the genus apart from the rest of the genera in the Gelidiales. He also confirmed the consistent lack of rhizines in this species and hence, the genus. Since then, a number of prominent researchers (Abbott 1999, Norris 1992b, Silva *et al.* 1996) have continued to recognise the Gelidiellaceae. Similarly, the merger of the Gelidiaceae and Gelidiellaceae has not been endorsed in this thesis. In analyses of chloroplast encoded ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) (Freshwater *et al.* 1994) and nuclear encoded small subunit rDNA (SSU) (Shimada *et al.* 1999, Shimada and Masuda 2000) gene sequences, where sequences from non-Gelidialean taxa have also been included, *Gelidiella* is resolved as the earliest diverging lineage within the Gelidiales (*Pterocladiella* was resolved basal to

Gelidiella in the *rbc*L phylogeny of Shimada *et al.* 1999, but had no branch support). According to Bailey and Freshwater (1997), the sequence divergence values between *G. acerosa* and other species in the Gelidiaceae (SSU: 2.0-2.9; *rbc*L: 12.7-15.4) and the long branch lengths leading to this species in trees indicate that, at the molecular level, *Gelidiella* is a very distinct taxon.

The classification of the Gelidiaceae has proven particularly difficult due to the variability in most aspects of the biology of its members and the prevalence of continuities in morphology. Ten morphological criteria have been applied to the Gelidiaceae to delimit genera and species within the family, some have been found to be of little taxonomic use, some have yet to be critically tested, and others are proving useful.

Holdfast type was first used as a generic criterion in the Gelidiaceae when Fan (1961) maintained the generic status of the monotypic genus *Suhria*, based on the presence of the discoid holdfast which was unique within the family. Perrone (1994) and Shimada *et al.* (1999) provided a new means to distinguish between genera in the Gelidiales, this being the type of secondary rhizoidal attachment produced. The authors found that *Gelidiella* produced unicellular independent attachments, *Pterocladia* and *Pterocladiella* produced peg-like attachments and the remaining genera (barring *Suhria* which has a discoid holdfast) formed brush-type attachments, but this criterion has yet to be critically tested.

The presence/absence of surface proliferations was used by Kylin (1956) to distinguish between *Ptilophora* and *Beckerella*. After considerable debate by numerous authors, Norris (1987) deemed the criterion inappropriate for generic distinction by showing the production of surface proliferations in the type species of both genera, and subsumed *Beckerella* into *Ptilophora*. However, Norris's proposal was not widely accepted.

The disposition of surface cortical cells was used by Akatsuka (1986a, 1986b) to establish *Pterocladiastrum* Akatsuka and *Onikusa* based on the shared occurrence of surface cortical cells in tetrads in the middle of the main thallus axis. However, Rodríguez and Santelices (1988) found tetrads of cortical cells to be common in species of *Gelidium* and *Pterocladia* (including *Pterocladiella*) in apical portions of the thallus. The disposition of surface cortical cells allowed some generic segregation between *Pterocladia* and *Gelidium* but exceptions were found to exist (Santelices and Hommersand, 1997).

Growth pattern was used by Okamura (1900) to delimit *Acanthopeltis* and *Yatabella* Okamura (two genera similar in vegetative and reproductive features) stating that *Acanthopeltis* exhibited sympodial growth whereas *Yatabella* exhibited monopodial growth as was found in other Gelidialean genera. Shimada *et al.* (1999) proposed that *Yatabella* be subsumed into *Acanthopeltis* based on congruence between three types of molecular data and by having shown that these genera shared a fundamentally similar growth pattern. Growth pattern was also used by Norris (1992a) to emend the description of *Onikusa*, highlighting its tendency to branch sympodially, after Rodriguez and Santelices (1988) cast doubt on the taxonomic status of this genus having shown its generic criterion to be artificial.

Life history was used by Guiry and Womersley (1993) to establish the monotypic genus *Capreolia* characterised by a biphasic life history in which the carposporophyte generation is absent, contrary to the triphasic life history of other Gelidialean algae. Similarly, Rico and Guiry (1997) described a diminutive alga *Gelidium maggsiae* Rico & Guiry distinguished by consistently monoecious gametophytic thalli. Monoecious plants had been reported elsewhere in the Gelidiaceae but their occurrence was always facultative.

Five morphological characters were proposed in the past for the separation of *Gelidium* from *Pterocladia*. Okamura (1934) proposed the use of rhizine distribution and the shape of medullary cells which proved to be of limited use due to changes in internal structure during the life of a frond (Dixon, 1958). Stewart (1976) proposed the use of basal branch bending at the point of branching of indeterminate branches in *Gelidium*, but this was found to occur in some species of *Pterocladiella* (Santelices and Hommersand, 1997). Rodriguez and Santelices (1988) proposed the use of apical architecture, but Santelices (1990) stated that this character was unreliable. The only criterion which was reliable in distinguishing between these genera was cystocarp structure (Santelices 1991a & 1991b, Hommersand and Fredericq 1996).

Freshwater *et al.* (1995) provided the first extensive *rbc*L phylogeny of the Gelidiales to develop hypotheses of the relationships among genera within the order. From their results it appeared that the classification of the order was not natural, since *Gelidium* and *Pterocladia* were paraphyletic with respect to other genera in the order. It became clear that taxonomic revisions within the genera would need to be made in order to develop a natural classification for the Gelidiales, reflecting evolutionary trends within the group. Rodríguez and Santelices (1996) described six types of medullary structure in a study of 21 Gelidiales species suggesting that this could be used to support the various *rbc*L clades resolved in the phylogeny of Freshwater *et al.* (1995). This criterion has yet to be critically tested. Congruence between molecular data and the structural variations of cystocarps in the Gelidiaceae was later found which led to the proposal of the genus *Pterocladiella* for four species formerly placed in *Pterocladia* and *Gelidiella* (Santelices and Hommersand 1997), and the subsequent transfer of additional species to the genus (Santelices 1997b, 1998, Thomas and Freshwater 2001). All these species are resolved in the same strongly supported *rbc*L clade and have a unilocular cystocarp with a cylindrical placental core of nutritive and gonimoblastic filaments either centrally positioned in the locule or attached on one of its sides to the floor of the cystocarp. New molecular data is expected to help elucidate other appropriate morphological characters that can be used as generic criteria to develop a natural classification of the Gelidiaceae.

External morphology in species of the Gelidiaceae has been shown to be significantly affected by biotic and abiotic environmental factors. External morphology such as branching pattern and the abundance of branches can be so strongly modified that seasonal growth forms are recognised in some species, for example *Pterocladia caerulescens* (Kützing) Santelices (Santelices, 1978). In studies of variation in external morphology of species in the field, the result has often been a reduction in the number of species recognised (Santelices 1976, 1977). DNA sequencing has contributed to a better understanding of species limits and the extent of morphological variation within a species (e.g. Shimada *et al.* 2000b), since regardless of environmental conditions, the variation (divergence) between sequences of closely related specimens is comparatively low. Freshwater and Rueness (1994) showed that intraspecific sequence divergence was generally less than 1% in a number of

Gelidiaceae taxa. Sequence divergence can thus be used as an indication of whether specimens are likely to be conspecific or not.

The economic utilization of the Gelidiaceae has stimulated systematic research on the family particularly in the last 30 years. Consequently, the regions of the world with high, or thoroughly researched, species diversity tend to coincide with regions that have an interest (either in a harvesting or processing capacity) in the commercial agarophyte industry. Chile, South Africa, the Mediterranean, the Philippines, and Japan are actively involved in harvesting agarophytes, whereas the USA, Japan and Europe are primarily involved in processing agarophytes. In South Africa, an industry has developed over the last four decades where two concessionaires (harvest rights-holders) harvest four species [*Gelidium abbottiorum* Norris, *G. pteridifolium* Norris, Hommersand & Fredericq, *G. capense* (Gmelin) Silva and *Onikusa pristoides* (Turner) Akatsuka] from the Eastern Cape Province coast (Fig. 1) where the largest agarophyte biomass in South Africa occurs. A recent evaluation of this industry (Tronchin *et al.*, 2003) found that it was sustainable and not likely to be resource limited at present levels of harvesting effort, and that it was entirely based on raw materials export. Systematic research on the South African Gelidiaceae gained momentum shortly after the establishment of the local industry and continues to date. The results of this research are hoped to lead to growth in the industry by providing a basis for other scientific research (e.g. mariculture) aimed at providing technological advancements to the industry.

The most significant contributions to the systematics of the South African Gelidiaceae since Kylin (1956) erected the genus *Beckerella* and described two new turf algae from the KwaZulu-Natal (KZN) coast (Fig. 1), were made towards the end of the 20th century. Norris (1987a, 1987b, 1990a, 1990b) and Norris *et al.* (1987) improved the understanding of the Gelidiaceae of the East coast, whilst Stegenga *et al.* (1997) researched the west coast species. Richard Norris focused his research on the flora of KZN since it had been poorly documented in the literature (Hommersand, 1986), and ultimately produced a detailed guide (Norris, 1992b) to the Gelidiaceae of KZN.

Fig. 1 – Map of South Africa illustrating the provinces and various collection locations.

By 1999, when this study began, the diversity of the South African Gelidiaceae stood at 21 species in six genera (Table 1). *Gelidium crinale* (Turner) Gaillon was reported to occur in South Africa by Delf and Michell (1921), but has not been mentioned since. Delf and Michell (1921) also reported the occurrence of *G. corneum* (Hudson) Lamouroux, though Papenfuss (*fide* Papenfuss notes) believed this to be a misapplied name, Silva *et al.* (1996) stating that this name should be applied to *G. sesquipedale* (Clemente y Rubio) Thuret. Papenfuss did not complete his review of South African *G. corneum* (Silva *et al.*, 1996) but thought the specimens he studied (from the Agardh collection) represented *G. amansii* (Lamouroux) Lamouroux, *G. crinale* and *G. capense.* A number of other *Gelidium* species names have been misapplied to South African collections. *Gelium amansii, G. asperum* (C. Agardh) Greville, *G. rigidum* (C. Agardh) Greville, and *G. cartilagineum* var. *setaceum* (C. Agardh) Grunow have been misapplied to South African *G. abbottiorum. Gelidium cartilagineum* (Linnaeus) Gaillon, *G. corneum, G. robustum* (Gardner) Hollenberg & Abbot and *G. versicolor* (Gmelin) Lamouroux have been misapplied to South African *G. capense* and *G. pteridifolium.*

Table 1. List of Gelidiaceae species occurring in South Africa prior to this study.

Genus	*species*
Beckerella	*B. helenae* (Dickinson) Fan & Papenfuss*
	B. hildebrandtii (Hauck) Kylin
	B rumpii (Dickinson) Papenfuss & Fan
	B. pinnatifida (J. Agardh) Kylin
Gelidium	*G. abbottiorum* Norris
	G. applanatum Stegenga, Bolton & Anderson
	G. arenarium Kylin
	G. capense (S.G. Gmelin) Silva
	G. crinale (Turner) Gaillon
	G. micropterum Kützing
	G. minusculum (Weber-van Bosse) Norris
	G. pteridifolium Norris, Hommersand & Fredericq
	G. reptans (Suhr) Kylin
Onikusa	*O. foliacea*
	O. pristoides
Pterocladia	*P. caespitosa* (Kylin) Norris
	P. caloglossoides (Howe) Dawson
Ptilophora	*P. diversifolia* (Suhr) Papenfuss
	P. rhodoptera Norris
	P. spissa (Suhr) Kützing
Suhria	*S. vittata* (Linnaeus) Endlicher

* placed in synonymy with *B. pectinata* (A. Gepp & E. Gepp) Fan & Papenfuss by Norris (1992)

Prior to this study the distribution of the Gelidiaceae of the South African west coast and KwaZulu-Natal province (Fig. 1) was well documented in Stegenga *et al.* (1997) and Norris (1992b) respectively. Numerous species checklists from various locations along the south coast and Eastern Cape coast existed, but a comprehensive review of the distribution of this family in South African had not been compiled.

Continued systematic research on the South African Gelidiaceae in this study was facilitated by a three-year collaboration with Dr. D.W. Freshwater from the University of North Carolina at Wilmington (UNCW) in the USA, who made molecular sequencing facilities available. This study also formed part of ongoing (since 1996) bilateral cooperation between phycologists at the University of Cape Town and the University of Ghent (RUG) in Belgium on a project entitled "Marine, shallow-water benthic macroalgae and echinoderms: biogeography and diversity in the transition zone of the tropical Indian Ocean to warm temperate southern Africa (KwaZulu-Natal, South Africa)". Fresh collections of South African Gelidiaceae were made from a number of locations along the northern Eastern Cape and KZN coast during 1999-2003, i.e. from Port Alfred, the coast between Cape Morgan and Kei Mouth, Breezy Point, Mntafufu, Port Edward, Palm Beach, Protea Banks, Aliwal Shoal, the coast between Isipingo and Durban, Zinkwazi, Mission Rocks, Sodwana, Mabibi, Black Rock, Kosi Bay, and Tiger Reef (Fig. 1).

The aims of this study were:

1. to continue ongoing systematic research aimed at approaching a natural and practicable classification of the Gelidiaceae by assessing the taxonomic status of the genera *Onikusa*, *Suhria*, *Ptilophora* and *Beckerella*, genera that are well represented along the South African coastline with respect to their global distribution;
2. to review the diversity and worldwide distribution of *Ptilophora*, a genus originally described from South Africa;
3. to document the occurrence of sponge-algal associations in *Ptilophora* thereby critically evaluate Norris's (1992b) hypothesis that the production of surface proliferations (the generic criterion) in members of this genus are induced by sponge epiphytes;

4. to elucidate the diversity of the South African Gelidiaceae, making use of molecular (*rbc*L and LSU gene sequence data) and morphological data, and finding congruence between these data;
5. to compile information on the vertical and horizontal distribution of the South African Gelidiaceae.

CHAPTER 1

A REASSESSMENT AND RECLASSIFICATION OF SPECIES IN THE GENERA *ONIKUSA* AKATSUKA AND *SUHRIA* J. AGARDH *EX* ENDLICHER (GELIDIALES, RHODOPHYTA) BASED ON MOLECULAR AND MORPHOLOGICAL DATA.

INTRODUCTION

Phylogenetic reconstructions based on a variety of DNA sequence data have shown that the red algal order Gelidiales is taxonomically problematic (Freshwater *et al.* 1995, Bailey and Freshwater 1997, Freshwater and Bailey 1998, Shimada *et al.* 1999). Separate and combined analyses of chloroplast encoded *rbc*L, nuclear encoded SSU and nuclear encoded LSU sequences have resolved four major clades within the Gelidiales that correspond to specific ontogenetic patterns of the female reproductive and cystocarp systems (Bailey and Freshwater 1997, Freshwater and Bailey 1998, Shimada *et al.* 1999). Three of these major clades correspond to the genera *Gelidiella* Feldmann *et* Hamel, *Pterocladia* J. Agardh and *Pterocladiella* Santelices *et* Hommersand. Species in the fourth major clade have a '*Gelidium*-type' female reproductive and cystocarp system. This major clade contains the majority of Gelidialean taxa and includes species in the genera *Acanthopeltis* Okamura *in* Yatabe (including *Yatabella* Okamura), *Capreolia* Guiry *et* Womersley, *Gelidium* Lamouroux, *Onikusa* Akatsuka, *Ptilophora* Kützing (including *Beckerella* Kylin) and *Suhria* J. Agardh *ex* Endlicher. Eight subclades (the species complexes of Freshwater *et al.* 1995) have been resolved within this major clade but they do not correspond to the currently accepted generic designations (Freshwater *et al.* 1995, Shimada *et al.* 1999); most notably the ordinal type, *Gelidium*, is paraphyletic. Revision of the generic assignment of some species is necessary to attain a phylogeny-based natural classification of the Gelidiales.

It may be argued that the strong molecular support for the subclades within the '*Gelidium*-type' female reproductive and cystocarp system clade should lead to the recognition of these subclades at the genus level. I believe that morphological/ontogenetic synapomorphies correlating with the molecular clades are required before recognition at the genus level is warranted. Numerous morphological criteria for distinguishing genera and species have been proposed for the Gelidiales including: 1] growth pattern (Okamura, 1900), 2] rhizine distribution (Feldmann and Hamel 1934, Okamura 1934), 3] presence/absence of surface proliferations (Kylin, 1956), 4] holdfast type (Fan 1961, Perrone 1994, Shimada *et al.* 1999), 5] basal bending of indeterminate branches (Stewart, 1976), 6] arrangement of surface cortical cells (Akatsuka 1981, 1986a, 1986b), 7] apical architecture (Rodríguez and Santelices, 1987), 8] life history (Guiry and Womersley 1993, Rico and Guiry 1997), 9] medullary structure (Rodríguez and Santelices, 1996), and 10] ontogenetic patterns of the female reproductive and cystocarp systems (Hommersand and Fredericq 1988, 1996; Santelices and Hommersand 1997). Only the ontogenetic patterns of female reproductive and cystocarp systems have proven reliable when critically examined. Medullary structure has not been thoroughly tested.

For example, detailed studies of the female reproductive system and cystocarp development by Santelices (1991a, 1991b) and Hommersand and Fredericq (1996) led to the proposal of the genus *Pterocladiella* for four species formerly placed in *Pterocladia* and *Gelidiella* (Santelices and Hommersand 1997). Subsequent examination of additional species has supported the establishment of *Pterocladiella* and resulted in the transfer and description of additional species in this genus (Santelices 1997b, 1998, Thomas and Freshwater 2001). Likewise, molecular analyses of species transferred to *Pterocladiella* strongly resolve them as a monophyletic clade supporting the distinction of this genus as a natural taxonomic group (Freshwater and Bailey 1998, Shimada *et al.* 1999, Thomas and Freshwater 2001, Freshwater *et al.* unpublished).

In this chapter, the development of a natural classification system for the Gelidiales is continued by revising the status of *Onikusa* and *Suhria*. This revision is based on past and new molecular and morphological analyses that include 8 previously unpublished *rbc*L sequences and a critical examination of medullary structure characteristics in a limited number of species. The relationship of *Onikusa foliacea* (Okamura) Norris and a reported turf form of *O. pristoides* (Turner) Akatsuka is also examined.

MATERIALS AND METHODS

Molecular analyses

Eight new and 29 previously published *rbc*L sequences were analysed in this study. Collection locations for the taxa from which they were generated are listed in Table 1.1. The new *rbc*L sequences came from specimens collected at field sites and dried using silica gel desiccant (Chase and Hills 1991). Total genomic DNA was extracted from 10-30 mg of tissue following the protocol of Hughey *et al.* (2001). Amplification and sequencing of *rbc*L was as described in Thomas and Freshwater (2001). Sequencing reactions performed using the Big Dye sequencing kit and protocol (Applied Biosystems, Foster City, CA) were analysed on either an ABI Prizm 377 or 3100 Genetic Analyzer (DNA Analysis Facility, Center for Marine Science, UNCW). The sequences of primers used in this study are presented in Freshwater and Rueness (1994). Sequence data were compiled and edited using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA). Characteristics of the aligned sequence data were determined using PAUP (v. 4.0b8, Swofford 2001) and MacClade (v. 4.0, Maddison and Maddison 2000). Refer to the CD-ROM attached for an *rbc*L gene data file including all the sequences analysed in this study.

Phylogenetic trees were generated using maximum parsimony, distance, and maximum likelihood methods. Parsimony trees were generated with a heuristic search scheme of 1000 random sequence additions, MULPARS, STEEPEST DESCENT, and the tree bisection reconnection (TBR) branch-swapping algorithm. Trees found in the initial search were then swapped to completion using, MULTREES, STEEPEST DESCENT and the nearest-neighbor-interchange (NNI) branch-swapping algorithm. Bootstrap analyses (Felsenstein 1985) consisted of 1000 replications of heuristic searches using simple sequence addition, MULTREES, STEEPEST DESCENT, and TBR branch swapping. For the distance analysis a neighbor-joining tree (Saitou and Nei 1987) was constructed from Tamura-Nei distances (Tamura and Nei 1993). Distance bootstrap analyses consisted of 1000 replications of neighbor joining tree construction with Tamura-Nei distances. Before performing maximum likelihood analyses the transition:transversion ratio (tn:tv) that maximized the log-likelihood value was calculated by plotting a range of tn:tv against the corresponding log-likelihoods for the distance tree. The resulting value (tn:tv = 3.2) was subsequently used with empirical base frequencies in 10 separate likelihood analyses of random sequence addition, MULTREES, and the TBR branch swapping algorithm. Maximum likelihood bootstrap values were calculated from 100 replications of random addition of sequences, MULTREES, and NNI branch swapping.

Table 1.1. Collection location for taxa included in *rbc*L sequence analyses. Sequences not previously published are indicated by an *.

Taxa	Collection Location
Gelidium allanii Chapman	Doubtless Bay, North Island, New Zealand[1]
G. americanum (Taylor) Santelices	Radio Island, Carteret Co. NC, USA[2]
G. canariensis (Grunow) Seoane-Camba	Puerto de la Cruz, Tenerife, Canary Islands[3]
G. capense (Gmelin) Silva	False Bay, Cape Peninsula, South Africa[4]
G. chilense (Montagne) Santelices *et* Montalva	Tongoy Bay, Coquimbo, Chile[5]
G. coulteri Harvey	Balboa Peninsula, Orange Co., CA, USA[2]
G. crinale (Turner) Gaillon	Masonboro Inlet, New Hanover Co., NC, USA[2]
G. crinale	Awhai Is., Hyogo Pref., Japan (Shimada *et al.* 1999)
G. floridanum Taylor	Sebastian Inlet, Indian River Co., FL, USA[2]
G. latifolium (Greville) Bornet *et* Thuret	Plouguerneau, Brittany, France[6]
G. microdonticum W.R. Taylor	Cahuita, Limón, Costa Rica[2]
G. micropterum Kützing	Kommetjie, Cape Peninsula, South Africa[4]
G. pacificum Okamura	Amatsukominato, Chiba Pref., Japan[7]
**G. pluma* Loomis	Hawai'i, Hawaiian Islands[8]
G. pulchellum (Turner) Kützing	Aramar, Asturias, Spain[9]
G. pusillum (Stackhouse) Le Jolis	Cancale, Brittany, France[9]
G. pusillum	Fedje, Hordaland, Norway[9]
G. 'pusillum'	Puerto de la Cruz, Tenerife, Canary Islands[9]
G. 'pusillum'	Praia de Peruibe, Estado de Sao Paulo, Brazil[10]
G. 'pusillum'	Solano Beach, San Diego Co., CA, USA[11]
G. 'reptans' (Suhr) Kylin	Natal, South Africa (culture #0962)[12]
G. rex Santelices *et* Abbott	Tongoy Bay, Coquimbo, Chile[5]
G. robustum (Gardner) Hollenberg *et* Abbott	Dana Point, Orange Co., CA, USA[2]
G. serrulatum J. Agardh	Mochimo, Sucre, Venezuela[2]
G. sesquipedale (Clemente) Thuret *in* Bornet *et* Thuret	Aramar, Asturias, Spain3
G. vagum Okamura	Jodogahama, Iwate Pref., Japan (Shimada *et al.* 1999)
**'Onikusa' foliacea* (Okamura) R.E. Norris	Port Edward, KwaZulu-Natal Prov., South Africa[13]
**'O.' foliacea*	East London, Eastern Cape Prov., South Africa[13]
**'O.' foliacea*	Breezy Point, Eastern Cape Prov., South Africa[13]
**'O.' japonica* (Harvey) Akatsuka	Keelung, Taiwan[14]
'O.' japonica	Shimoda, Shizuoka Pref., Japan (Shimada *et al.* 1999)
'O.' sp.	GenBank submission by Shimada *et al.*
'O.' pristoides (Turner) Akatsuka	False Bay, Western Cape Prov., South Africa[11]
**'O.' pristoides*	Port Edward, KwaZulu-Natal Prov., South Africa[13]
**'O.' pristoides* "turf form"	Kidds Beach, Eastern Cape Prov., South Africa[13]
Ptilophora scalarimosa (Kraft) Norris	Bulusan, Sorsogon Province, Luzon, Philippines[15]
Suhria vittata (Linnaeus) J. Agardh	Kommetjie, Western Cape Prov., South Africa[11]
**S. vittata*	Lüderitz, Namibia[16]

Samples for DNA extraction collected by: [1]W. Nelson, [2]D.W. Freshwater, [3]J. Rico, [4]J. Bolton, [5]M.E. Edding, [6]J. Cabioc'h & M.H. Hommersand, [7]M. Yoshizaki, [8]K.J. McDermid, [9]culture of J. Rueness & S. Fredricksen, [10]M. Cordeiro-Marino, [11]M.H. Hommersand, [12]culture of Rico & Guiry, [13]E.M. Tronchin, [14]S.M. Lin, [15]L. Liao, [16]A.T. Critchley.

Morphological observations

Medullary structure characters defined in Rodríguez and Santelices (1996) were revisited using nine species in the Gelidiaceae. Observations were made of the medullary structure of *Suhria vittata* (Linnaeus) Endlicher (Oudekraal, RSA), *Onikusa foliacea* (East London, RSA), *O. pristoides* (Glencairn, RSA), *O. japonica* (Harvey) Akatsuka (Keelung, North Taiwan), *Gelidium micropterum* Kützing (Gelncairn, RSA), *G. microdonticum* Taylor (Cahuita, Costa Rica), *G. capense* (S.G. Gmelin) Silva (Glencairn, RSA), *G. coulteri* Harvey (Stillwater Cove, Pacific Grove, California, USA) and *G. chilense* (Montagne) Santelices *et* Montalva (Tongoy Bay, Coquimbo, Chile). Silica gel dried and preserved specimens (fixed in 5-10% Formalin-seawater at the time of collection) where used. Dried specimens were hydrated in seawater, or 5% Formalin-seawater. Following the method described by Rodríguez and Santelices (1996), paradermal, longitudinal and cross sections were cut by hand or with a freezing microtome from apical tips 0.5 cm in length. All specimens were stained with 1% aniline blue stain and mounted in 50% Karo^{TM} solution. Specimens were observed and photographed using a Nikon Coolpix 990 digital camera (Nikon Corp., Tokyo, Japan) mounted on a Leitz Dialux 20 EB compound microscope (E. Leitz, Inc., Wetzlar, Germany).

RESULTS

Molecular analyses

A *rbc*L data set of 37 taxa was analyzed in this study. The first 67 base pairs (bp) of the 1467 bp gene were excluded from all analyses because a majority of the analyzed taxa were missing data for these sites. Of the remaining 1400 sites, 433 were variable and 312 were parsimony informative. The data set had an overall AT base bias, a tn:tv of 3.4 and a bias of 324 pyrimidine transition substitutions to 207 purine transition substitutions. Based on these findings, the Tamura-Nei correction was used when calculating all distances.

The topologies derived from all three tree-building methods were nearly identical. Parsimony analyses resulted in three minimal trees of 973 steps and consistency indices (CI) of 0.43 (Fig. 1.1). The distance and maximum likelihood topologies differed only in the relationship of *Gelidium chilense* to *G. rex* Santelices *et* Abbott.

Samples of *Onikusa pristoides*, *O. foliacea* and *Suhria vittata* were resolved within a strongly supported clade (bootstrap proportion [BP] = parsimony [P] 95, distance [D] 100, maximum likelihood [L] 84) referred to as the '*Suhria* species complex' by Freshwater *et al.* (1995). Sequences of *S. vittata* from Kommetjie, South Africa and Lüderitz, Namibia varied by 0.48 %. *Gelidium micropterum* had a strongly supported sister relationship to *Suhria vittata* (BP = P98, D96, L92). Distances between sequences of *Onikusa pristoides* from Port Edward, Kidds Beach and False Bay, South Africa ranged from 0.0 to 0.50%. The turf form sample from Kidds Beach had an identical sequence to the sample from Port Edward. Similarly, distances between sequences of *O. foliacea* from Port Edward, East London and Breezy Point, South Africa ranged from 0.0 to 0.22%. Samples of *O. pristoides* (BP = P100, D100, L98) and *O. foliacea* (BP = P99, D100, L94) were strongly supported as distinct monophyletic clades that are sister in the *rbc*L tree (BP = P100, D100, L100). Sequences for *O. japonica* from Japan and Taiwan were strongly resolved as a monophyletic clade (BP = P100, D100, L100) within a clade separate from that which includes *O. pristoides* and *O. foliacea*. The strongly supported clade containing the *Onikusa* and *Suhria* species also include species of *Gelidium* (Fig. 1.1).

Morphological analyses

No differences in medullary cell dimensions or organization were found between cells hydrated with seawater or a 5% Formalin-seawater solution. Similarly, no differences were found when comparing sections mounted in seawater to sections mounted in a 50% Karo^{TM} solution.

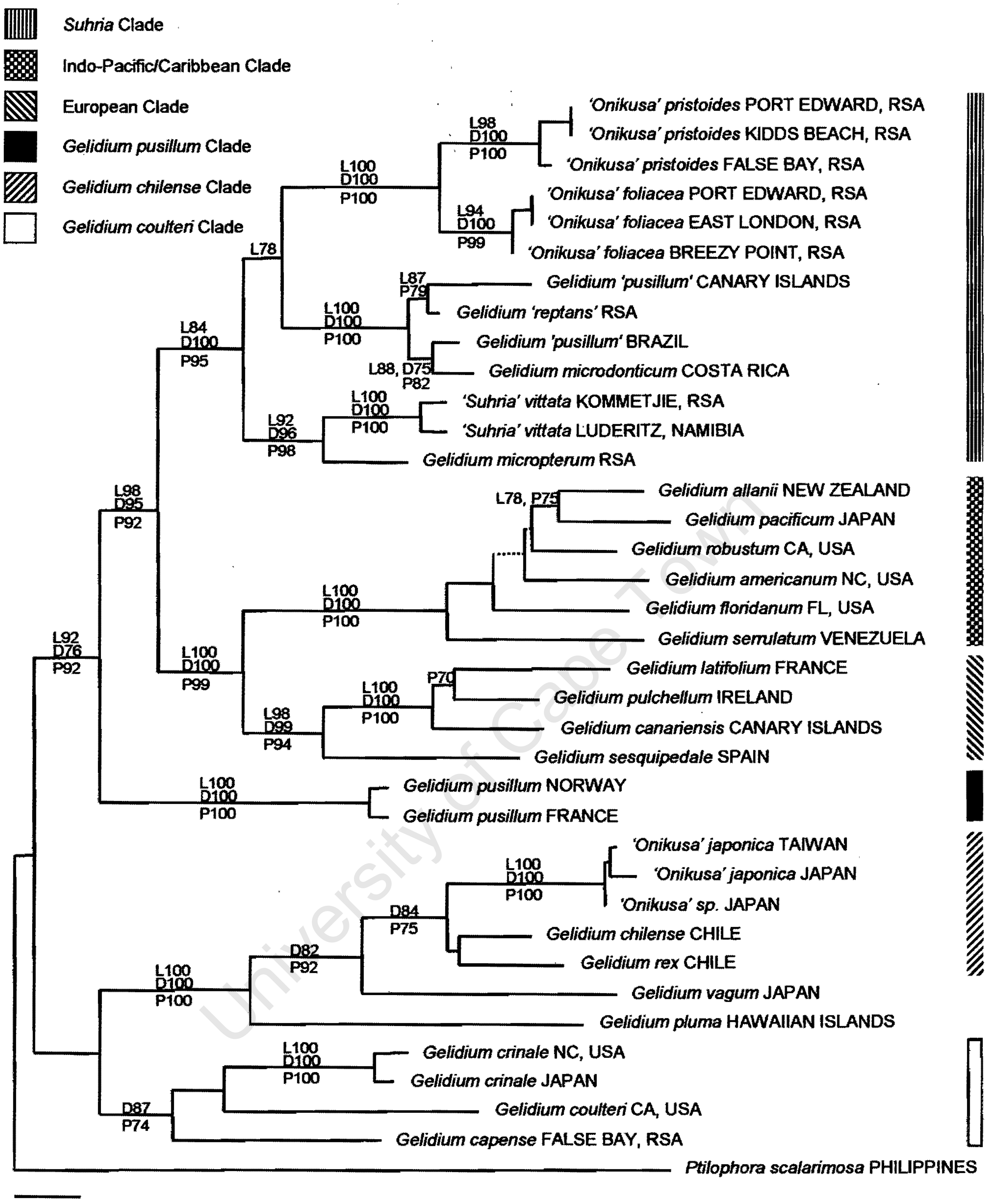

Fig. 1.1. One of six minimal parsimony trees (L=973, CI=0.43) resulting from analyses of *rbc*L sequence data from 37 Gelidiales taxa. Branches not present in all minimal trees are stippled. Bootstrap proportion values for Parsimony (P), Distance (D) and maximum likelihood (L) analyses are presented on branches when greater than 70. Scale = 10 changes.

Paradermal sections of *Suhria vittata* revealed a loose mesh of narrow, elongated medullary cells with lateral and terminal connections that ran in various directions (Fig. 1.2). Medullary cells were 2-9 μm in diameter (with within-cell variation of ±1.5 μm) and 35-185 μm in length.

Paradermal sections of *Onikusa pristoides* revealed a very regular medullary structure composed of closely associated cells oriented parallel to one another. The regular structure was enhanced by the presence of numerous lateral H-shaped intercellular connections between adjacent cells (Fig. 1.3). Medullary cells were 2-7 μm in diameter (with within-cell variation of 1-2.5 μm) and 24-109 μm in length. Rhizines were abundant in the inner cortex, especially in the alae, but absent in the medulla of the midrib.

Gelidium coulteri had closely apressed cylindrical cells with rhizines concentrated in the inner cortex (Fig. 1.4). *Gelidium capense* had predominately cylindrical cells, but flattened cells were also encountered. *Onikusa japonica* had mostly cylindrical cells in subapical sections with flattened ribbon-like cells occurring subcortically and scattered throughout the medulla. *Gelidium chilense* had mostly cylindrical cells subapically with relatively few scattered subcylindrical cells (Fig. 1.5). *Gelidium capense* and *Onikusa japonica* had rhizines concentrated in the inner cortex whereas rhizines occurred throughout the medulla of *Gelidium chilense.*

All nine species had a medullary structure that could be described as a mesh, differing in various degrees of looseness and regularity. *Onikusa pristoides*, *Gelidium coulteri* and *G. chilense* had the tightest and most regular medullary structure. Evidence of varying degrees of twisting in medullary cells was found in all the species. The presence of H-shaped intercellular connections (Fig. 1.2) was noted in *Onikusa pristoides*, *O. japonica*, *Gelidium micropterum*, *G. capense*, *G. coulteri*, *G. chilense* and *Suhria vittata* (though not common in the latter).

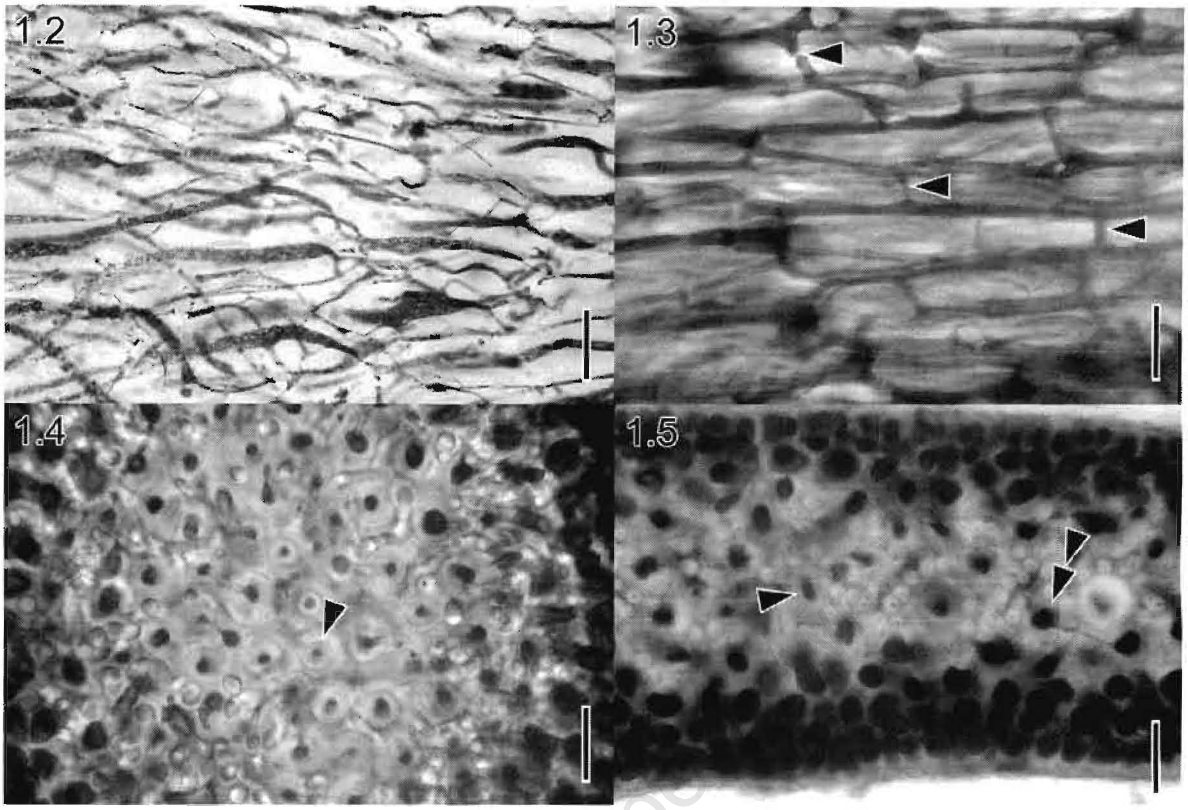

Fig. 1.2. Subapical paradermal section of *S. vittata* showing a loose mesh of medullary cells. Scale = 50 µm. Fig. 1.3. Subapical paradermal section of *O. pristoides* showing a regular network of medullary cells and H-shaped intercellular connections (arrowheads). Scale = 20 µm. Fig. 1.4. Subapical cross section of *G. coulteri* showing cylindrical medullary cells and rhizines concentrated in the inner cortex (arrowhead). Scale = 20 µm. Fig. 1.5. Subapical cross section of *G. chilense* showing cylindrical cells (double arrowhead) and few scattered subcylindrical medullary cells (single arrowhead). Scale = 20 µm.

DISCUSSION

Molecular Analyses

Analyses of *rbc*L sequence data support the recognition of *Onikusa foliacea*, *O. japonica*, *O. pristoides* and *Suhria vittata* as discrete species. Percent sequence divergence between specimens of these species were low (≤0.5%) despite the considerable geographical distance between sample locations. The *rbc*L phylogeny presented here (Fig. 1.1) is congruent with the results of previous *rbc*L analyses (Freshwater *et al.* 1995, Shimada *et al.* 1999) in resolving a number of well-supported subclades within the major clade of species with the '*Gelidium*-type' female reproductive and cystocarp system. Two of the three species currently placed in *Onikusa* are resolved along with *Suhria* *vittata* and five species of *Gelidium* within what has been referred to as the '*Suhria* clade' or '*Suhria* species complex.' The remaining species of *Onikusa, O. japonica,* is resolved within a separate group of species that is referred to as the '*Gelidium chilense* clade' (Fig. 1.1). As in all previous analyses with *rbc*L and other DNA sequences (Freshwater *et al.* 1995, Bailey and Freshwater 1997, Freshwater and Bailey 1998, Shimada *et al.* 1999, Thomas and Freshwater 2001) *Gelidium* is paraphyletic with respect to *Onikusa* and *Suhria.*

Medullary cell structure

Rodríguez and Santelices (1996) described six types of medullary structure in a study of 21 Gelidiales species. Medullary cell organization, morphology and dimension, as well as the distribution of rhizines defined these types. The characterization of medullary types was restricted to apical and subapical portions of erect axes, with the rationale that in this region cells are becoming a distinctive tissue still unmodified by age and environment and should not be variable in form and shape. Some of the defined medullary structure types were observed in taxa that had previously been resolved in specific species clades by *rbc*L analyses (Rodríguez and Santelices 1996: Table 1.3). In the present study, medullary structure type was examined in multiple species from the *rbc*L-defined *Suhria, Gelidium coulteri* and *G. chilense* species clades to determine if the *Onikusa pristoides, Gelidium coulteri* and *G. chilense* medullary types are synapomorphic for these species clades respectively.

The '*Onikusa pristoides* type' was described as a loose mesh of narrow, elongated medullary cells with lateral and terminal connections that run in various directions. Subapical cells were 50-90 µm long and 2-4 µm in diameter. The '*Gelidium coulteri* type' was described as a loose composition of flattened (ribbon-like) cells running parallel to the axis, with various degrees of undulation and twisting. Rhizines occurred throughout the medulla of both types. Medullary cells of the '*Gelidium chilense* type' were described to be cylindrical or subcylindrical apically and elliptical or compressed subapically, exhibiting varying degrees of twisting, with H-shaped intercellular connections in paradermal view.

The medullary structure of *Suhria vittata* was found to fit the general description of the '*Onikusa pristoides* type', but it has much larger cell dimensions than the dimensions defining this type. The medullary structure of the *O. pristoides* specimen observed in this study was very regular and did not fit the description of the '*Onikusa pristoides* type'. Neither did the cell dimensions and the distribution of rhizines agree with the description of this type. Figure 3.5c p. 25 in Carter (1986) also shows a very regular medullary structure with wide intercellular spaces, parallel rows of cells and H-shaped intercellular connections. Interspecific variation in medullary structure was also found among *O. foliacea*, *Gelidium micropterum* and *G. microdonticum*, three other species resolved in the '*Suhria* clade'. The lack of a consistent medullary structure type in the species of the *Suhria* clade demonstrates that these characters can not be used as a morphological synapomorphy for the clade.

Given that *Onikusa japonica* is resolved in an *rbc*L clade with *Gelidium chilense*, the medullary structures of *Onikusa japonica*, *Gelidium chilense* and two species of the related '*Gelidium coulteri* clade', *G. coulteri* and *G. capense*, were investigated. The medullary structures of all four species disagreed with the description of the '*Gelidium coulteri* type', given the presence of both cylindrical and flattened (or subcylindrical) cells and an even distribution of rhizines throughout the medulla. The medullary structure of *Onikusa japonica* did not fit the '*Gelidium chilense* type' due to the occurrence of both cylindrical and flattened ribbon-like cells subapically. The *G. chilense* examined here did not agree entirely with the '*Gelidium chilense* type' as cylindrical cells were found throughout the length of the apical tip, even up to 1 cm below the apex. Neither the *G. coulteri* nor *G. chilense* type medullary structures defined by Rodríguez and Santelices (1996) adequately define the taxa studied here.

Medullary structure type, rhizine distribution and cell dimension were found to be inconsistent between species in the 3 examined *rbc*L-defined clades. Therefore these characters can not be used as synapomorphies for these species clades. Additional study of these characters is needed to determine their potential utility as synapomorphies for other species and species clades.

Onikusa

Akatsuka (1986b) erected the genus *Onikusa* for two species formerly included in *Gelidium*, *Onikusa pristoides* from South Africa (type species) and *O. japonica* from East Asia. Norris (1992a) later described a third species, *O. foliacea*, for a taxon from Japan and South Africa.

Akatsuka (1986b) assigned members of the Gelidiales to groups based on various combinations of morphological characters. *Onikusa pristoides* and *O. japonica* were removed from *Gelidium* and placed in the '*Suhria*' group (including *Suhria*, and *Porphyroglossum* Kützing) based on the shared occurrence of surface cortical cells in tetrads in the middle of the main thallus axis (Akatsuka 1986b). *Onikusa*, *Porphyroglossum* and *Suhria* were treated as separate genera due to other macro- and micro-morphological differences. Algal taxonomists however, have not uniformly accepted *Onikusa* (Stegenga *et al.* 1997, Yoshida 1998).

Rodríguez and Santelices (1988) found tetrads of cortical cells to be common in species of *Gelidium* and *Pterocladia* (including *Pterocladiella*) in apical portions of the thallus and that they can occur elsewhere along the axis as well. The occurrence of cortical cells in tetrads is indicative of areas where cell division is still common, and the distance between cells and cell shape have not been modified by growth (Santelices 1988, Norris 1992a). Akatsuka (1986b) supported the use of surface cell morphology to delimit this genus with various vegetative diagnostic characters of the middle region of the main axis. These characters however, show much overlap with other genera such as *Ptilophora*. One of the characters used, the distribution of proliferations or serrulae, is environmentally variable and the presence of surface proliferations can be modified by injury or epiphytic load (Santelices, 1990).

Norris (1992a) emended the description of *Onikusa* to highlight a combination of characters in *O. pristoides* and *O. foliacea* he believed were important in delimiting this genus, i.e. the tendency to branch ramisympodially (thought to be a primitive form of ramisympodial branching with the major branches usually being produced adventitiously and emerging from the midrib) and the extensive indeterminate system of prostrate branches. Norris was unsure of the presence of this combination of characters in *O. japonica*, but examination of specimens shows that it has the same tendency to produce major branches adventitiously from the midrib and also possesses an extensive system of prostrate branches. However, *O. japonica* normally exhibits irregularly to alternately bipinnate branching and there is no mention of ramisympodial branching in the descriptions of this species (Harvey 1859, Akatsuka 1986b). Likewise, the ramisympodial tendency of branching in *O. pristoides* appears to be the exception rather than the rule and is most often an artifact caused by injury to the dominant axis stunting its growth and allowing a lateral to overtop it and assume secondary dominance. Overtopping due to injury of dominant axes is not uncommon and is seen throughout *Gelidium*. The system of indeterminate prostrate branches is also present in most *Gelidium* species though not always as extensive as in *Onikusa pristoides*. Consequently, this combination of characters is not unique to *Onikusa*.

Morphological characters as well as *rbc*L sequence data indicate that *Onikusa japonica* is not closely related to the other *Onikusa* species. The sporophyte generation of *O. japonica* always produces tetraspores (Akatsuka, 1983) whereas that of *O. pristoides* produces bispores (Carter, 1985), and the characteristic serrulae of *O. pristoides* do not occur in *O. japonica* (Akatsuka, 1983). Analyses of the *rbc*L sequence data resolve *Onikusa* species in two different strongly supported clades that are well separated in the *rbc*L phylogeny (Fig. 1.1). The polyphyly of species currently included in *Onikusa* and the lack of morphological synapomorphies for any combination of *Onikusa* species indicates that it is an artificial assemblage and that it should be reincorporated into *Gelidium*.

Norris (1992a) proposed the name *Onikusa foliacea* to describe an alga forming a mostly monospecific turf, documented to occur along the central and southern KwaZulu-Natal coastline in South Africa. Carter (1986) mentioned a similar turf alga that occurred in his study sites along the Eastern Cape Province coastline of South Africa. Carter suggested that it was an ecotype of *O. pristoides* or at most a variety. It has since been unclear whether the turf investigated by Carter was *O. foliacea* or whether there is indeed a turf form of *O. pristoides*. Carter mentioned that both the large and turf thalli had tooth-like serrulae along the margins of their flattened fronds, a character particular to *O. pristoides* and not *O. foliacea* (Norris, 1992b).

The turf form specimen of *O. pristoides* from Kidds Beach in Eastern Cape Province was found to have an identical *rbc*L sequence to one of the large form *O. pristoides* specimens from Port Edward in KwaZulu-Natal Province. By contrast, the *O. pristoides* and *O. foliacea* specimens from Port Edward varied by >2.0%. These data as well as the resolution of separate monophyletic clades corresponding to *O. pristoides* and *O. foliacea* in phylogenetic analyses clearly shows that a turf form (ecotype) of *O. pristoides* exists. This taxon is distinguishable by its midrib in well-developed blades and tooth-like marginal serrulae in contrast to *O. foliacea*, which has entire to erose to crispate margins.

Suhria

Suhria is currently a monotypic genus. *Suhria vittata* has had a long history of nomenclatural changes, having been placed in *Sphaerococcus* (C.A. Agardh 1822: 233), *Phyllophora* (Greville 1830: 135), *Gelidium* (Kützing 1843: 407), *Dawsonia* (Bory *in* Bélanger 1834:171) and even referred to as a different genus and species on occasion, i.e. *Fucus ornatus* Thunberg (Thunberg 1794: 181 - in part; 1823: 753 - in part), *Fucus ciliatus* Thunberg (Thunberg 1794: 181 - in part; 1823: 753 - in part; Esper 1797-1799), *Fucus caulescens* Gmelin (Gmelin 1768: 173) and *Delesseria caulescens* Lamouroux (Lamouroux 1813: 38)(Anderson, 1994). J. Agardh (1842) first proposed *Suhria* as a nomen nudum based on *Phyllophora vittata* Greville 1830, after which, Endlicher (1843) provided the first diagnosis of the genus. During the eighteenth century seven other species were included in *Suhria*, but all were later removed. The diagnosis of *Suhria* in Endlicher (1843) was heavily weighted on the reproductive proliferations issuing from the blade and was very general in its description. The structures described occur throughout the Gelidiaceae. Agardh (1823) described the holdfast of *S. vittata* (as *Sphaerococcus vittata* C. Agardh) as a "radix callosa", or callose root, hinting at its discoid nature. Fan (1961) maintained the generic status of *Suhria* based on the presence of the discoid holdfast being unique within the Gelidiales, but this may alternatively be considered simply a species specific character state.

Suhria vittata is resolved within a strongly supported clade of *Gelidium* species in *rbc*L analyses (Fig. 1.1), and the presence of a discoid holdfast is the only character state unique to this taxon. Given this information, the maintenance of *Suhria* as a monotypic genus is not warranted and it should be reincorporated into *Gelidium*.

Synapomorphies for a '*Suhria* clade'?

If a morphological synapomorphy for the '*Suhria* clade' were found the clade could be recognized at the genus level, in which case the name *Suhria* would have priority and be applied to all of the species within the clade. Of the characters that have been proposed in the past, only bispore production and the nature of gonimoblast/nutritive filament cell fusions remain as possible morphological synapomorphies for the '*Suhria* clade.'

Bisporangia have been reported to occur in four of the eight species that are resolved in this clade, i.e. *Onikusa foliacea*, *O. pristoides*, *Suhra vittata* and *Gelidium micropterum*. The species for which the presence of bisporangia has not been reported may not have been studied with the specific objective of looking for bisporangia.

Freshwater *et al* (1995) suggested that the presence of a large post-fertilization fusion cell could be another possible synapomorphy. It was stated that Fan (1961) and Hommersand and Fredericq (1990) demonstrated that the fusion of the carpogonium with adjoining cortical cells results in the formation of a large fusion cell, a feature that could be a synapomorphy for the *Suhria* complex. However, Fan (1961) reported the occurrence of fusion cells in all members of the Gelidiales that were investigated: In addition to *S. vittata*, he found them to occur in *Gelidium robustum* (Gardner) Hollenberg *et* Abbott (as *G. cartilagineum* var. *robustum* Gardner), *Onikusa japonica* (as *G. japonicum* (Harvey) Okamura), *Ptilophora subcostata* (Okamura) Norris (as *Beckerella subcostata* (Okamura) Kylin) and *P. pinnatifida* (J. Agardh) Norris (as *B. pinnatifida* (J. Agardh) Kylin). Although Fan (1961) reported that cystocarp development was very similar in the species he studied, there may be slight differences in the development of the fusion cell and gonimoblast that he did not document. Hommersand and Fredericq (1990) reported that a possible difference between *Gelidium* and *Suhria* cystocarp development is that terminal gonimoblast cells either fuse specifically with the terminal cells of the nutritive filaments, as in *Gelidium pteridifolium* Norris, Hommersand *et* Fredericq, or randomly with terminal and intercalary cells, as in *S. vittata*. It is most probable that this difference is only species specific and it must be studied in additional species before being considered as a synapomorphy for the '*Suhria* clade'.

Santelices (1999) has recently reported 4 different patterns of carpospore production in a study of 12 Gelidiales species. These patterns varied in the size of the fusion network, shape of the carposporangia-initiating gonimoblast cell, and the arrangement and way carposporangia are produced. One of the examined species was *Onikusa pristoides* but the carposporangia-production pattern ascribed to this species was also found in *Gelidium chilense*, *G. rex* Santelice *et* Abbott, and *G. pluma* Loomis. These four species are resolved in three separate species clades in molecular analyses (Fig. 1.1, and Freshwater unpublished analyses). Although the *Onikusa pristoides* pattern of carposporangia production is not a synapomorphy for the *Suhria* clade, further investigation of the characteristics described by Santelices (1999) may reveal synapomorphies for other *Gelidium* species clades.

CONCLUSION

Maintaining *Suhria* and *Onikusa* as genera has been shown to be untenable. There are two possible synapomorphies for the '*Suhria* clade' that could lead to the continued use of *Suhria*, however, the presence of either synapomorphy in the *Suhria* clade and absence in the other *rbc*L clades has yet to be confirmed. Before the *Suhria* clade could be recognized at the generic level, however, the clades occurring as sister groups to the *Suhria*-clade in the *rbc*L phylogeny would have to be recognized as genera in order to maintain monophyly in the tree. There are taxonomic problems in these basal clades that have yet to be resolved, therefore, it would seem reasonable to work with the information at hand to continue the process of revising the classification of the Gelidiales so that it is practicable. Since *Gelidium* is paraphyletic with respect to *Suhria* and *Onikusa*, these two genera should be reincorporated into *Gelidium*. The following taxonomic changes are thus proposed:

Suhria vittata will revert to *Gelidium vittatum* (Linnaeus) Kützing (1843: 407). A new combination is here proposed for *Suhria vittata* forma *lacerata*.

Gelidium vittatum (Linnaeus) Kützing forma *laceratum* (Grunow) Tronchin comb. nov.
Basionym: *Suhria vittata* forma *lacerata* Grunow, 1867. *Reise der österreichischen Fregatte Novara um die Erde in den Jahren* 1857, 1858, 1859...*Botanischer Theil. Erster Band. Sporenpflanzen*: 81.
Type locality: St. Paul Island, sin typo.

Onikusa pristoides will revert to *Gelidium pristoides* (Turner) Kützing (1843: 407). A new combination is here proposed for *Onikusa foliacea*.

Gelidium foliaceum (Okamura) E.M. Tronchin comb. *et* stat. nov.
Basionym: *Gelidium pusillum* forma *foliaceum* Okamura, 1934. *J. Imp. Fish. Inst.* (Tokyo Fish. Inst.) 29: 38,39; pl. 17; figs 3-4; pl. 31.
Type locality: Japan, on barnacles at Shisô-dima, Seto, Prov. Kii, Okamura s.n. (iso. LD)
Synonym: *Onikusa foliacea* (Okamura) R.E. Norris, *Taxonomy of Economic Seaweeds with Reference to Some Pacific and Western Atlantic Species, Vol. III*. 1992: 169, figs 7-10 (including Indian Ocean Record)

- Norris, The marine red algae of Natal, South Africa: order Gelidiales (Rhodophyta). *Mem. Bot. Surv. S. Africa* 61: 21, figs 10,11.

Onikusa japonica will revert to *Gelidium japonicum* (Harvey) Okamura, (1901: 57-60).

CHAPTER 2

A RE-EVALUATION OF THE GENERA *BECKERELLA* AND *PTILOPHORA* (GELIDIALES, RHODOPHYTA) BASED ON MOLECULAR AND MORPHOLOGICAL DATA.

INTRODUCTION

Holmes (1896) proposed a separate section of the genus *Ptilophora* Kützing (Gelidiales, Rhodophyta) containing species characterised by flattened fronds without proliferations on the surface. Kylin (1956) erected the genus *Beckerella*, the absence of proliferations on the thallus surface being the major character distinguishing it from the genus *Ptilophora*. Akatsuka (1987) considered that the presence of a large-celled inner cortex, together with the absence of surface proliferations was the most effective criterion for distinguishing *Beckerella* from all other Gelidiales. For some time previously there had been disagreement among phycologists as to the appropriateness of using surface proliferations as a generic character in the Gelidiales, Kützing (1847) having placed particular emphasis on the vegetative structure and the presence of surface proliferations in delimiting *Ptilophora*. Schmitz (1894) did not consider the presence/absence of proliferations to be a good generic character and returned *Ptilophora prolifera* (Harvey) J. Agardh to *Gelidium*, where Harvey (1855) had originally placed it. Papenfuss (1940) and Fan (1961) attached major importance to the presence of surface proliferations in defining *Ptilophora*, but Norris (1987a) merged it with *Beckerella* after discovering surface proliferations in the type species of *Beckerella*, *B. pinnatifida* (J. Agardh) Kylin. Norris (1987a) regarded the four-layered vegetative construction of *Ptilophora* (including *Beckerella*) species, as had been previously alluded to by other phycologists (Kützing 1847; Agardh 1876; Holmes 1896; Fan 1961; Huvé 1962; Kraft 1976; Akatsuka & Masaki 1983, Akatsuka 1987), to be the major genus-defining feature, rather than proliferations, which he regarded as probably a facultative response to epiphytic sponges.

The reclassification of *Beckerella* by Norris (1987a) has not been widely accepted (Murase *et al.* 1989; Silva *et al.* 1996; Trono 1997; Barreto *et al.* 1997; Kraft *et al.* 1999). Akatsuka (1987) and Athanasiadis (1987), who recognised *Beckerella*, were probably unaware of Norris's (1987a) proposed change as all three papers were published in the same year. Silva *et al.* (1996) provisionally accepted both genera and proposed the new combination *Beckerella pterocladioides* (Andriamampandry) P. Silva for the species originally described as *Ptilophora pterocladioides* Andriamampandry.

Norris's (1987a) examination of surface proliferations included only one *Beckerella* and two *Ptilophora* species. The four-layered vegetative construction of *Ptilophora* and *Beckerella* species has been illustrated in line drawings of varying quality but only one photomicrograph of this character has been published (Akatsuka & Masaki 1983). In this chapter the supposed diagnostic characters of *Beckerella* and *Ptilophora* are reassessed based on molecular analyses and more extensive morphological investigations of external morphology and vegetative structure in numerous species attributed to both genera. These are the first molecular analyses to include data from species classified within *Ptilophora* prior to its merger with *Beckerella*. Photomicrographs are also provided illustrating the characteristic four-layered vegetative construction in five additional species.

MATERIALS AND METHODS

Molecular analyses

Specimens of fresh material used for *rbc*L and LSU sequence analyses were collected from field sites and dried in silica gel desiccant (Chase & Hills, 1991). Small pieces of dried herbarium specimens were utilised for a limited number of taxa for which field collections were not available. Specimen collection locations are listed in Table 2.1. Total genomic DNA was extracted following the protocols of either Freshwater & Rueness (1994) or Hughey *et al.* (2001). Amplification and sequencing of chloroplast-encoded *rbc*L and a portion of the nuclear-encoded large-subunit ribosomal RNA gene (LSU) was done as described in Thomas & Freshwater (2001). The sequences of primers used in this study are those of Freshwater & Rueness (1994) and Freshwater & Bailey (1998). Sequence data were compiled and aligned using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and MacClade (v. 4.0, Maddison & Maddison 2000). Characteristics of the aligned sequence data were determined and phylogenetic analyses were performed using MacClade and PAUP (v. 4.0b8, Swofford 2001). Refer to the CD-ROM attached for *rbc*L and LSU gene data files including all the sequences analysed in this thesis.

Two different molecular data sets were analysed in this study. The *rbc*L data set included sequences from 36 taxa (29 previously published). Due to missing data at the 5' end of many *rbc*L sequences the first 67 sites of the 1467 bp gene were excluded from the analyses. The LSU data file included 24 taxa (16 previously published) in an alignment of 1159 total sites. This section of the LSU gene contains portions of the D and E major branches of the secondary structure model presented for *Palmaria palmata* (Linnaeus) Kuntze (Auwera *et al.* 1998), and has been analysed in previous studies of the Gelidiales (Freshwater & Bailey 1998; Freshwater *et al.* 1999; Rico *et al.* in press). Phylogenetic trees were generated from these data files using maximum parsimony, distance and maximum likelihood methods.

Parsimony analyses of the *rbc*L data set consisted of a two-part heuristic search. Initially 1000 random sequence additions using MULTREES, STEEPEST DESCENT, and nearest-neighbour interchange (NNI) branch swapping algorithm (but keeping only 40 trees each step) were performed. Trees found in this initial search were then swapped to completion using MULTREES, STEEPEST DESCENT and tree bisection-reconnection (TBR) branch swapping algorithm. Parsimony analyses of the LSU sequence data were performed with the branch-and-bound search algorithm. Parsimony bootstrap analyses consisted of 2000 replications of 10 random sequence additions, MULTREES and TBR (*rbc*L), or 1000 replications of branch-and-bound searches (LSU). Distance trees were generated using neighbour-joining tree building with Tamura-Nei distances. The Tamura-Nei correction was chosen because both data sets had an unequal frequency of bases and a purine-purine or pyrimidine-pyrimidine transition bias. Distance bootstrap analyses consisted of 2000 replications of neighbour-joining tree building with Tamura-Nei distances. Maximum likelihood analyses were done using transition:transversion ratios of 2.60 (*rbc*L) and 2.25 (LSU) and empirical base frequencies. Ten random additions of sequences with MULTREES and TBR branch swapping were used for likelihood searches of both data sets. Likelihood bootstrap analyses consisted of 250 (*rbc*L) or 300 (LSU) replications of one random sequence addition with MULTREES and TBR branch swapping. Additionally, quartet-puzzling analyses of 1000 puzzlings using the same model parameters as in likelihood searches were performed for both the *rbc*L and LSU sequence data files.

A combined *rbc*L + LSU data file including the ten analysed *Ptilophora* and *Beckerella* species plus, *Capreolia implexa* Guiry & Womersley as an outgroup, was used for constrained analyses. Branch-and-bound parsimony searches were performed with and without constraining the resulting tree to include separate monophyletic clades for *Ptilophora* and *Beckerella* species.

Morphological observations

The occurrence of surface proliferations was investigated using a Wild stereo dissecting photomicroscope. To determine whether there were differences between the surface proliferations of putative species of *Ptilophora* and *Beckerella*, the external morphologies were noted. Comparisons were also made of the internal structures of surface proliferations in *Ptilophora diversifolia* (Suhr) Papenfuss, *P. prolifera*, *Beckerella scalaramosa* Kraft and *B. hildebrandtii* (Hauck) Kylin by cutting transverse and longitudinal sections of their apical and basal portions.

In order to determine whether the four tissue layers were unique to species of *Ptilophora* and *Beckerella* but absent in all other Gelidiaceae, transverse sections were cut from second-order laterals in *Ptilophora*, *Beckerella*, and large thalli of other genera, and from proximal regions of primary axes in small and turf-like species (Table 2.1).

Changes in vegetative structure with thallus age were investigated by cutting transverse sections in apical (within 2 mm of the branch apex), subapical (about 5 mm from the branch apex) and proximal (second-order branches and main axes) regions of indeterminate axes. Observations of this nature were made in *P. prolifera*, *Beckerella subcostata* (Okamura) Kylin, *B. pectinata* (A. Gepp & E.S. Gepp) Fan & Papenfuss and *B. scalaramosa*.

All sections were cut by hand from pressed or silica-dried material. Sections were stained with 1% aniline blue stain, fixed in 50% Karo™ solution, and observed under a Zeiss compound photomicroscope.

Table 2.1. Collection locality of taxa included in morphological analyses (Morph) and molecular analyses (Mol) of *rbc*L and LSU sequences.

Species	Collection location	Morphology
Beckerella hildebrandtii	Mombassa, Kenya (holotype)	Morph
B. hildebrandtii	Tiger Reef, Bhanga Neck, KwaZulu-Natal, South Africa	Mol
B. mediterranea	Cape Matapan, S. Peloponnesus, Greece (isotype)	Morph/Mol
B. pectinata	Maroubra Bay, New South Wales, Australia (holotype)	Morph
B. pinnatifida	Protea Banks, KwaZulu-Natal, South Africa	Morph
B. pinnatifida	Sharks Bay, Port Alfred, Eastern Cape, South Africa	Mol
B. pterocladioides	Mokala, Madagascar (holotype)	Morph/Mol
B. rumpii	Richards Bay, KwaZulu-Natal, South Africa (holotype)	Morph
B. scalaramosa	Bulusan, Luzon, Philippines	Morph/Mol
B. subcostata	Chiba, Japan	Morph
B. subcostata	Fujisawa, Kanagawa, Japan	Mol
B. sp.[1]	Protea Banks and Palm Beach, KwaZulu-Natal, South Africa	Morph/Mol
Ptilophora diversifolia	Protea Banks, KwaZulu-Natal, South Africa	Morph/Mol
P. prolifera	Cawaramup Bay, Western Australia, Australia	Morph
P. rhodoptera	Protea Banks, KwaZulu-Natal, South Africa	Morph/Mol
P. spissa	'Omsamculo', S. KwaZulu-Natal, South Africa	Morph
P. sp.	Protea Banks, KwaZulu-Natal, South Africa	Morph/Mol
Acanthopeltis japonica	Chikura-Town, Chiba, Japan	Morph
Capreolia implexa	Port Philip Bay, Victoria, Australia	Morph/Mol
Gelidium asperum	Warnambool, Victoria, Australia	Morph
G. abbottiorum	Breezy Point, Eastern Cape, South Africa	Morph
G. canariensis	Puerto de la Cruz, Tenerife, Canary Islands	Mol (*rbc*L only)
G. capense	Storm's River Mouth, Eastern Cape, South Africa	Morph
G. capense	False Bay, Cape Peninsula, South Africa	Mol (*rbc*L only)
G. caulacantheum	Porirua Harbor, North Island, New Zealand	Morph/Mol
G. chilense	Tongoy Bay, Coquimbo, Chile	Morph
G. coulteri	Pacific Grove, California, USA	Morph
G. coulteri	Balboa Peninsula, Orange Co., California, USA	Mol (*rbc*L only)
G. crinale	Masonboro Inlet, North Carolina, USA	Mol
G. divaricated	Tokawa, Choshi, Chiba, Japan	Mol (*rbc*L only)
G. floridanum	Sebastian Inlet, Florida, USA	Mol
G. floridanum	Praia de Peruibe, Estado de Sao Paulo, Brazil	Morph
G. japonicum	Cyoshi-City, Chiba, Japan	Morph
G. japonicum	Keelung, Tawain	Mol
G. latifolium	Portstewart, Co. Londonderry, Northern Ireland	Morph
G. latifolium	Plouguerneau, Brittany, France	Mol
G. microdonticum	Cahuita, Limon, Costa Rica	Mol (rbcL only)
G. micropterum	Clovelly, False Bay, Cape Peninsula, South Africa	Morph
G. micropterum	Kommetjie, Cape Peninsula, South Africa	Mol (rbcL only)
G. pacificum	Matsugahana, Amatsukominato, Chiba, Japan	Mol (rbcL only)
G. pluma	Hilo, Hawai'i, Hawaiian Islands	Mol (rbcL only)
G. pristoides	Clovelly, False Bay, Cape Peninsula, South Africa	Morph
G. pristoides	False Bay, Cape Peninsula, South Africa	Mol
G. pteridifolium	Isipingo, Kwazulu-Natal, South Africa	Morph
G. pulchellum	Fanore, Co. Clare, Ireland	Morph
G. pusillum	Ambletusa, France	Morph
G. pusillum	Cancale, Brittany, France	Mol (rbcL only)
G. pusillum	Fedje, Norway	Mol
G. rex	Tongoy Bay, Coquimbo, Chile	Mol (rbcL only)
G. sesquipedale	Biarritz, France	Morph
G. sesquipedale	Aramar, Asturias, Spain	Mol
G. serrulatum	Mochimo, Venezuela	Mol
G. vagum	Iwate, Japan (Shimada *et al.* 1999)	Mol (rbcL only)
G. vittatum	Oudekraal, Cape Town, South Africa	Morph
G. sp.	Piha, North Island, New Zealand	Mol (rbcL only)
Pterocladia lucida	Owhiro Bay, South Wellington, New Zealand	Morph/Mol
Pterocladiella bartlettii	Cahuita, Limon, Costa Rica	Morph
P. bartlettii	Port Aransas, Texas, USA	Mol
P. caerulescens	Sandy Beach, Oahu, Hawaiian Islands	Morph
P. capillacea	Cottesloe Reef, Perth, Western Australia, Australia	Morph
P. capillacea	Torre a Mare, Bari, Italy	Mol
P. melanoidea	Mallorca, Spain	Mol

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[1] This specimen was misidentified when collected and originally published as *Ptilophora pinnatifida* (Freshwater *et al.* 1995).

RESULTS

Molecular Analyses

The analysed *rbc*L data set included 36 species and 1400 nucleotide sites. 516 sites (36.9%) were variable and 407 (29.1%) were parsimony-informative. This data set had an unequal frequency of bases (A=30.9%; C=16.7%; G=21.2%; T=31.2%), a transition:transversion ratio of 2.6, and a bias towards pyrimidine-pyrimidine transitions (61.7%). Parsimony searches resulted in three minimal trees of 1632 steps (Fig. 2.1). Trees derived from distance; maximum likelihood and quartet puzzling analyses differed only in the position of some individual *Gelidium* species and clades of *Gelidium* species. A clade consisting of all *Ptilophora* and *Beckerella* species was resolved with strong bootstrap support in all analyses. The topology within the *Ptilophora/Beckerella* clade was identical in parsimony and maximum likelihood trees and varied only by the position of *B. pterocladioides* in distance and quartet puzzling trees. *Beckerella* was paraphyletic with respect to *Ptilophora* in all analyses of *rbc*L data.

The analysed LSU data set included 24 taxa and 1159 sites. Insertion-deletion mutations (indels) occurred at 11 sites within the alignment, but no single indel included more than two sites. Due to the small size of indels, sites coded as gaps were treated as a fifth base in parsimony analyses. The LSU alignment included 129 variable sites (11.1%), of which 91 (7.9%) were parsimony-informative. Base use was uneven (A=23.9%; C=21.6%; G=30.9%; T=23.6%), and there was a transition:transversion ratio of 2.25, with a small bias towards purine-purine transitions (54.5%). Parsimony searches of the LSU data set found one minimal tree of 202 steps (Fig. 2.2). Tree topologies resulting from distance, maximum likelihood and quartet puzzling analyses did not differ significantly from the parsimony trees. In all types of searches a monophyletic *Ptilophora/Beckerella* clade was moderately to strongly supported by bootstrap analyses. *Beckerella* was paraphyletic with respect to *Ptilophora* in all analyses.

Parsimony analysis of the combined *rbc*L + LSU data file (11 taxa, 2559 sites, 310 variable sites and 127 parsimony informative sites) for *Ptilophora* and *Beckerella*

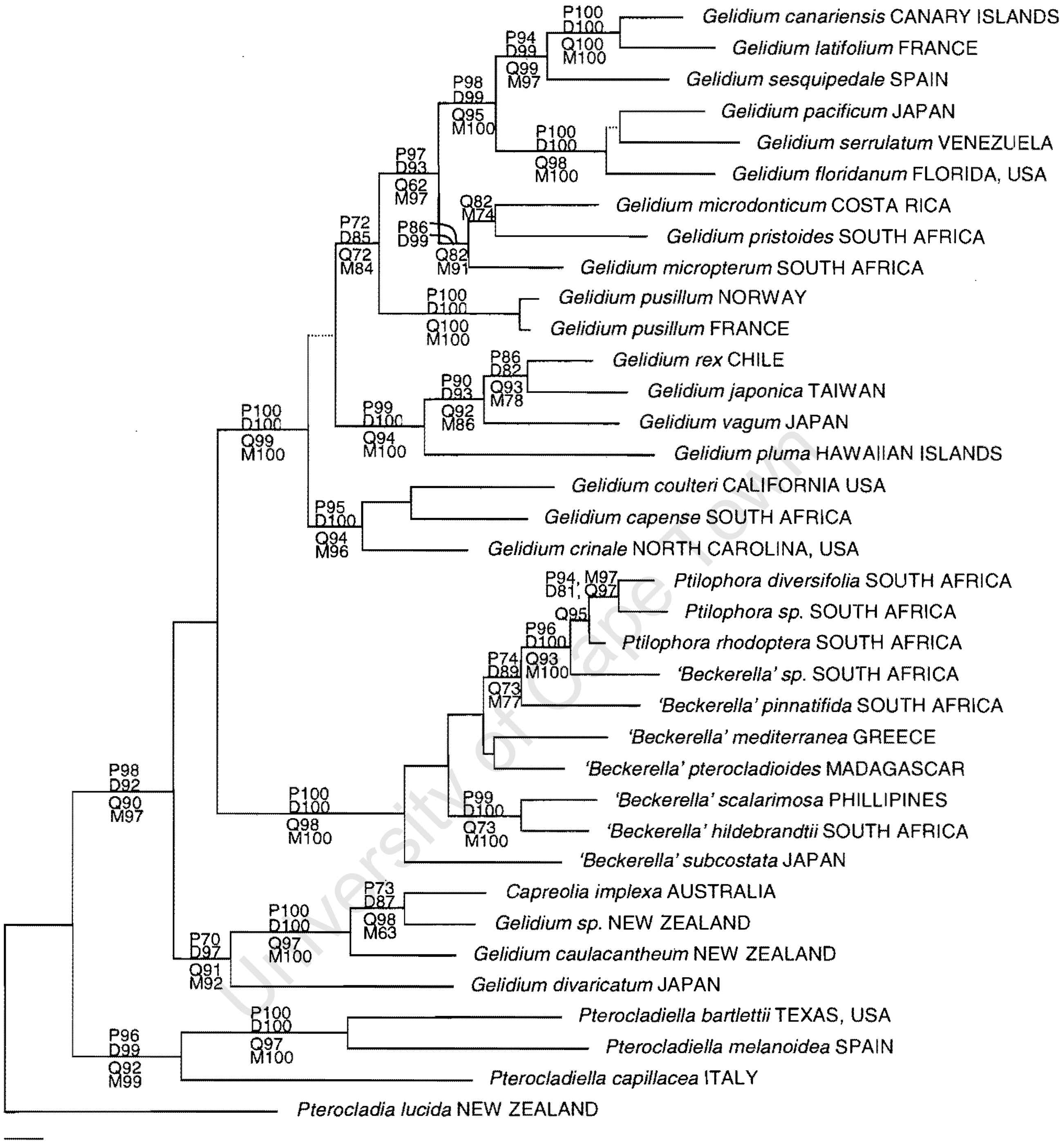

Fig. 2.1. One of three minimal trees (length [L] = 1632; consistency index [CI] = 0.316) resulting from parsimony searches of *rbc*L sequences from 36 Gelidiales species. Branches not present in all minimal trees are represented by dashed lines. Bootstrap support (%) and quartet puzzling reliability values are given for branches when greater than 70 (P = parsimony; D = distance; Q = quartet puzzling; L = maximum likelihood). Scale = 10 changes.

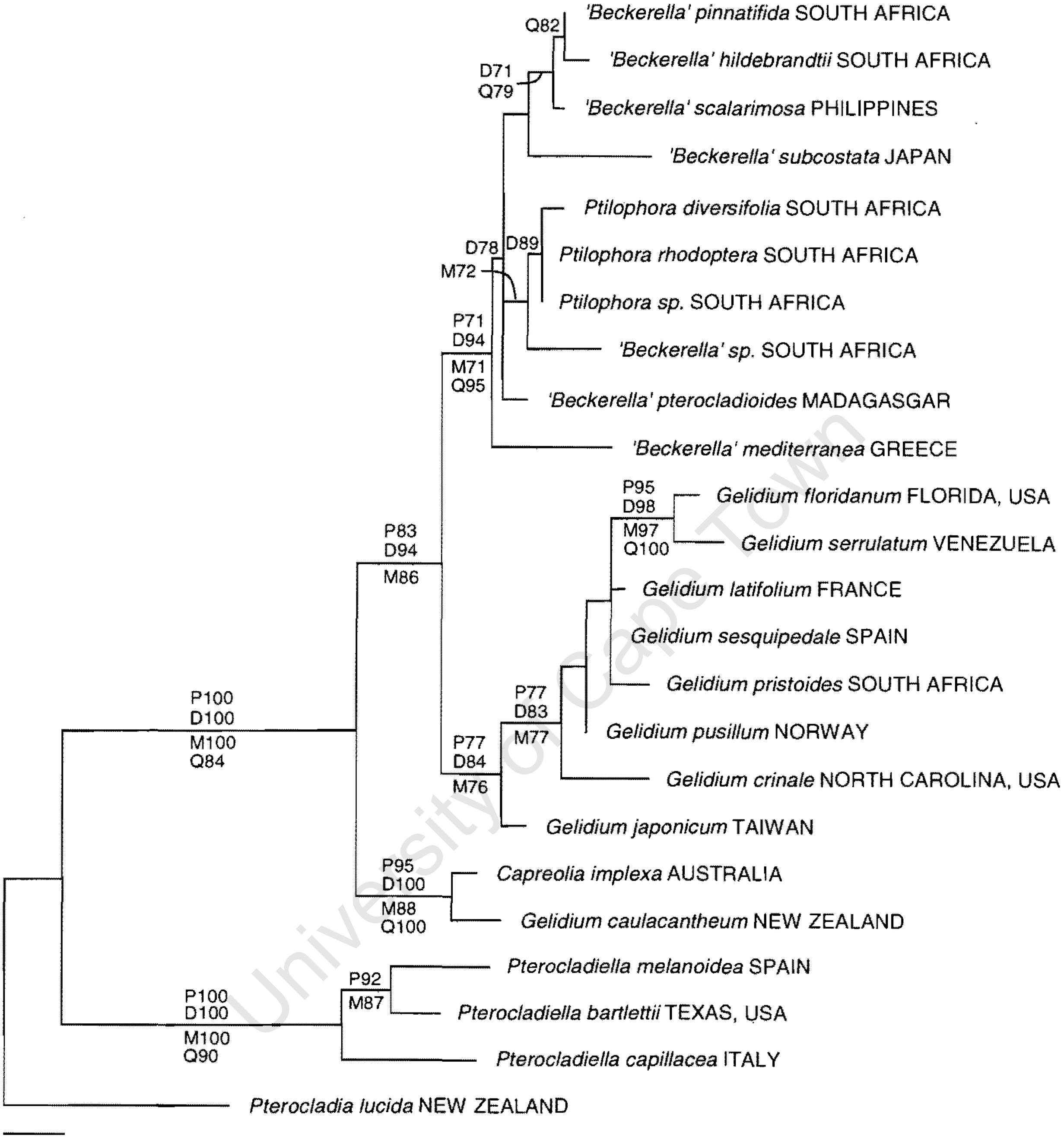

Fig. 2.2. One of three minimal trees (L = 202; CI = 0.639) resulting from parsimony searches of partial LSU sequences (1159 sites) from 24 Gelidiales species. Bootstrap support (%) and quartet puzzling reliability values are given for branches when greater than 70 (P = parsimony; D = distance; Q = quartet puzzling; L = maximum likelihood). Scale = 5 changes.

species resulted in a single minimal tree of 252 steps (Fig. 2.3). Parsimony analysis of this same data file that was constrained to resolve separate monophyletic *Ptilophora* and *Beckerella* clades resulted in a single minimal tree of 279 steps. This is a penalty to parsimony of 27 steps, which is 9.68 % of the unconstrained parsimony tree length.

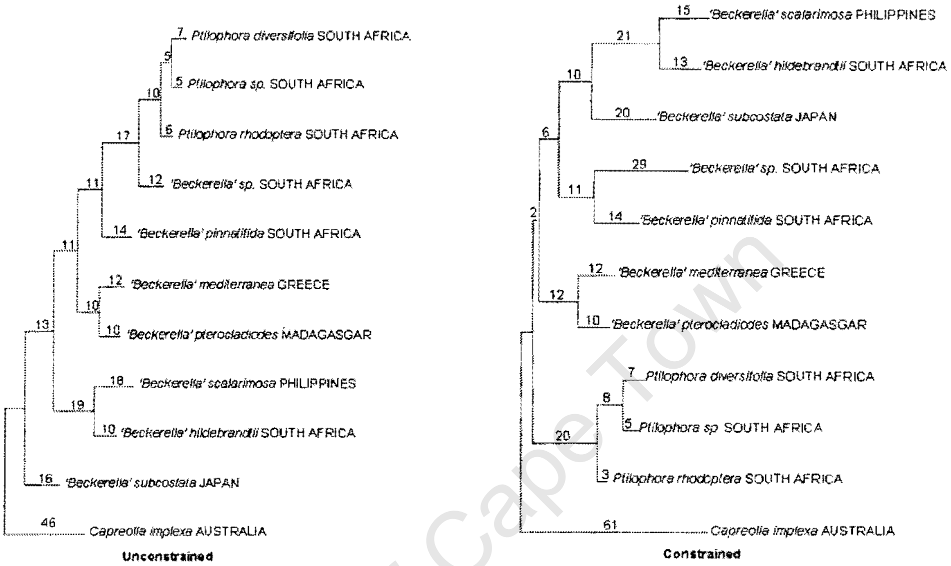

Fig. 2.3. Minimal trees resulting from two separate parsimony searches of combined *rbc*L and LSU sequence data from 10 species within the *Ptilophora/Beckerella* clade and *Capreolia implexa* as an outgroup. One search was unconstrained (tree length = 252, CI = 0.504; informative sites only) and the other was constrained (tree length = 279, CI = 0.455; informative sites only) to resolve separate monophyletic clades for *Ptilophora* and *Beckerella*. Branch lengths are indicated above internodal branches.

Morphological Observations

Nine of the 14 examined species of *Ptilophora* and *Beckerella* were found to have surface proliferations [*viz. Ptilophora spissa* (Suhr) Kützing, *P. diversifolia* (Fig. 2.4), *P. rhodoptera* Norris, *P. prolifera*, *Ptilophora sp.*, *Beckerella pinnatifida* (Fig. 2.5), *B. hildebrandtii* (Fig. 2.6), *Beckerella sp.* and *B. scalaramosa*]. Three species, *Ptilophora spissa*, *P. diversifolia* and *P. prolifera* were similar in that they had a very extensive covering of surface proliferations. *Ptilophora spissa* produced scale-like and ligulate proliferations on the midrib and crenate frond margins, *P. diversifolia*

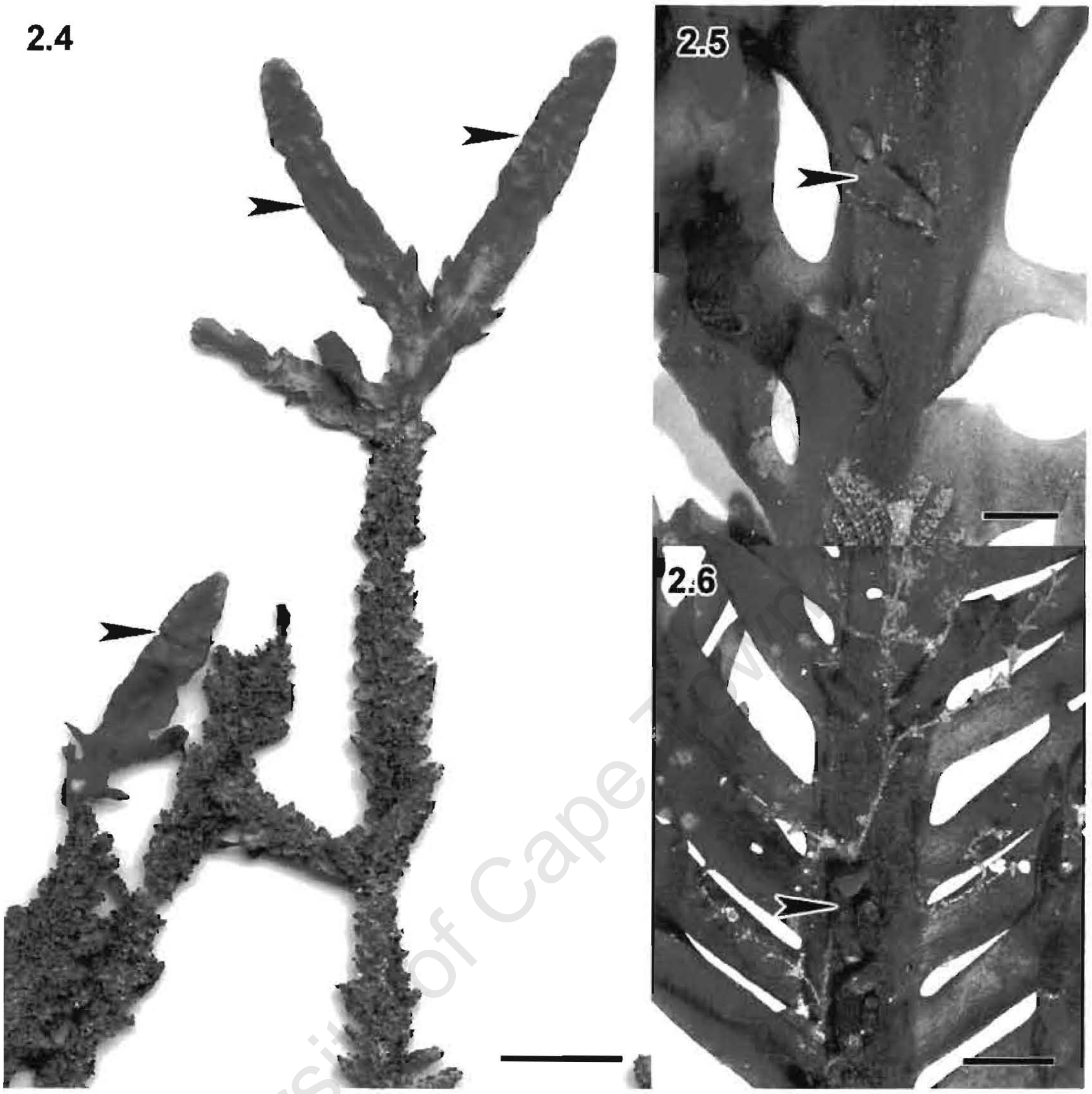

Fig. 2.4. *Ptilophora diversifolia*. Branches covered in a sponge epiphyte. Only the distal fronds have smooth surfaces (arrowheads) free from surface proliferations and sponge. Scale = 1 cm. Fig. 2.5. *Beckerella pinnatifida*. A branch with two surface proliferations (arrowhead) issuing from the midrib. Scale = 1 mm. Fig. 2.6. *Beckerella hildebrandtii*. A main axis with surface proliferations (arrowhead) along its length. Scale = 1 mm.

(Fig. 2.4) and *P. prolifera* produced proliferations as described in Norris (1987a) and Womersley & Guiry (1994) respectively, and *Ptilophora sp.* produced ligulate, often pinnate, proliferations arranged in an irregular fashion on the surface of the frond. *Ptilophora rhodoptera* produced short cylindrical and ligulate proliferations either centred or just to the side of the midrib, although these were relatively few in number (three in total on a 4 cm long frond for example). Similarly, proliferations occurring on *Beckerella pinnatifida* (Fig. 2.5) and *B. hildebrandtii* (Fig. 2.6) were rare although many surface proliferations have been reported from sponge-covered specimens of *B.*

pinnatifida (Norris 1987a). Surface proliferations in *B. pinnatifida* were mostly cylindrical while those in *B. hildebrandtii* were ligulate or sometimes pinnate. Only one of eight specimens of *Beckerella sp.* was found to produce surface proliferations, these consisting of two compressed and pinnately branched enations. Two types of proliferations were found on *B. scalaramosa*, those produced distally on the thallus and those in proximal regions. The former, observed in one specimen, were subcylindrical to cylindrical, sometimes branched, from 4-20 mm in length, and all borne about 5 cm from the base of a second-order branch. They were less pigmented than the bearing branch as they had a single-layer outer cortex. The latter were minute (± 550 µm long, ± 150 µm in diameter at their base), only produced on the basal 1–1.5 cm of the main axes, and had an acute apex with a dividing apical cell.

A four-layered vegetative structure was clearly visible in proximal transverse and longitudinal sections of the older and more developed surface proliferations from species of both *Ptilophora* and *Beckerella* (Fig. 2.7). Short proliferations (≤ 1mm in length) consisted almost entirely of cortical cells and lacked medullary filaments, as did apical parts of longer proliferations.

2.7

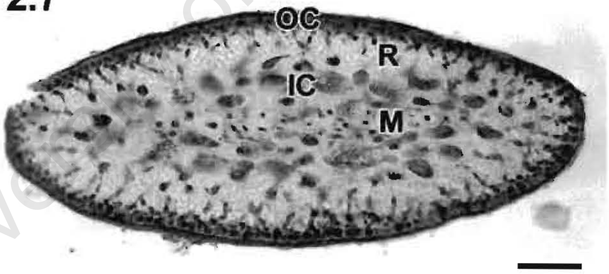

Fig. 2.7. *Beckerella hildebrandtii*. Transverse section through the base of a small surface proliferation. (OC) Outer cortex; (R) rhizine layer; (IC) inner cortex; (M) medulla. Scale = 50 µm

In longitudinal and transverse sections of second order branches, all the species of *Ptilophora* and *Beckerella* had a vegetative structure comprised of four different concentric tissue layers (Figs. 2.8-2.12). The outer cortex consisted of one layer of tiny, heavily pigmented cells, which were most often quadrangular or elongated anticlinally. Beneath this layer were rhizines that were directed longitudinally towards the base of the thallus (Fig. 2.8). The rhizines were so numerous, tightly packed and contained between the peripheral layers of pigmented outer cortical cells and the inner cortex that they constituted a veritable tissue (Figs. 2.9-2.12). Filaments (generally four to five cells long) of pigmented, angular to elongate cortical cells traversed the band of rhizines, tending to be anticlinally arranged, conferring a bundled appearance to the rhizines (Fig. 2.11). The inner cortex generally consisted of two to four layers of large cells, spherical to elliptical in shape and periclinally arranged when viewed in transverse sections (Figs. 2.9-2.12), but could even be six cell layers thick as was found in some specimens of *P. diversifolia*. The core was comprised of a filamentous medulla consisting of long, thick-walled cells that were more or less cylindrical and orientated longitudinally to the thallus axis (Fig. 2.9). Scattered within the medulla of all species except for *B. pectinata*, *B. scalaramosa* and *P. prolifera* were thinner walled cells with much larger lumens, approximating the appearance of the inner cortical cells. Rhizines were absent from the inner cortex and medulla of most studied sections. Transversely oriented rhizines encircling the inner cortical cells were observed in *P. prolifera*, *B. subcostata*, *B. scalaramosa* (Fig. 2.10), *B. pectinata*, *B. pterocladioides* and *B. biserrata* (Børgesen) Fan & Papenfuss. In nine of the twelve species of *Ptilophora* and *Beckerella* examined, rhizines were also found to occasionally traverse the inner cortex and intertwine with the medullary filaments. The rhizines were normally scarce when this occurred but they were found to be numerous in the inner cortex and medulla of *B. pectinata* and *B. pterocladioides*. The original descriptions and illustrations of *B. irregularis* Akatsuka & Masaki (Akatsuka & Masaki, 1983) and *B. biserrata* (Børgesen, 1943) were referred to since no material of these two species was available for sectioning. Both species were found to have a vegetative structure that agreed with the pattern described above.

The distinctness of the four tissue layers varied with the age of the thallus section examined. In apical transverse sections of *Ptilophora prolifera*, *Beckerella subcostata*, *B. pectinata* and *B. scalaramosa* the outer cortex was one cell layer thick. The cortical filaments were disorganised and not yet anticlinally arranged, but nevertheless conferred a bundled appearance to the dense band of rhizines beneath. The border between the rhizine band and inner cortex was distinct. The inner cortex comprised the majority of the vegetative structure and consisted of variably sized ovoid to elliptical cells that were bigger and thicker-walled than the outer cortical

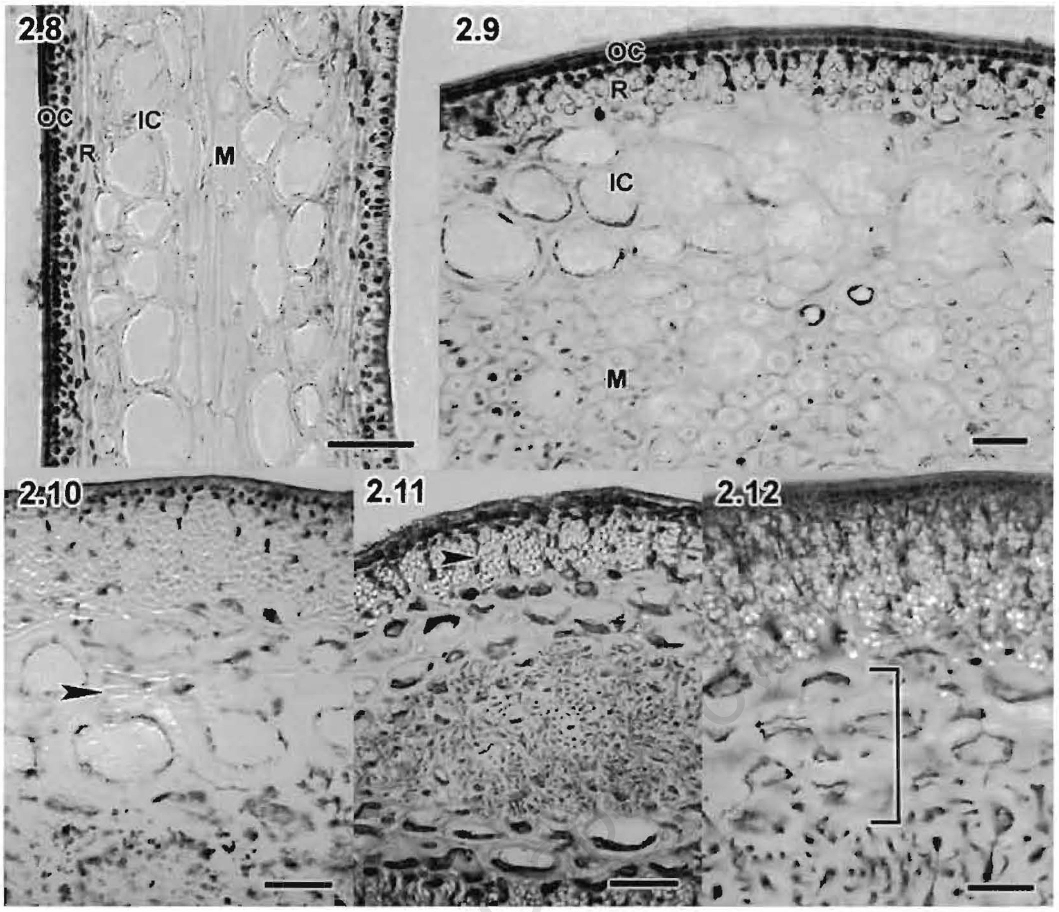

Fig. 2.8. *Beckerella subcostata*. Longitudinal section of second-order branch. (OC) Outer cortex; (R) rhizine layer; (IC) inner cortex; (M) medulla. Scale = 50 µm. Fig. 2.9. *Beckerella subcostata*. Transverse section of second-order branch. (OC) Outer cortex; (R) rhizine layer; (IC) inner cortex; (M) medulla. Scale = 25 µm. Fig. 2.10. *Beckerella scalaramosa*. Transverse section of second-order branch with transversely oriented rhizines (arrowhead) in the inner cortex. Scale = 50 µm. Fig. 2.11. *Beckerella pinnatifida*. Transverse section of second-order branch with anticlinal filaments of pigmented cortical cells (arrowhead) traversing the rhizine layer. Scale = 50 µm. Fig. 2.12. *Ptilophora diversifolia*. Transverse section of second order branch with an inner cortex four cell-layers thick (bracketed). Scale = 50 µm

cells. The medulla was indistinct apically. Rhizines were only found scattered throughout the core in *B. scalaramosa* and *Ptilophora prolifera* and running transversely between cells in *P. prolifera*. There was no midrib at this point in the thallus. Subapically, the rhizine layer was thicker and the filaments of angular pigmented cells traversing the rhizine layer consequently more distinct. In some sections, a medulla became discernible at a point coinciding with the formation of a midrib. Isolated clusters of rhizines occurring mostly towards the margins in flattened blades were sometimes found in the medulla. The four-layered vegetative structure was present in all transverse sections of second-order branches. In transverse sections of proximal parts of the main axis the outer cortex of small, pigmented cells was a few cell layers thick. The rhizine band was considerably thicker and the anticlinal rows of pigmented cortical cells were no longer distinct. Numerous rhizines were directed transversally in the inner cortex, obscuring the elliptical cells in this region. Rhizines were also more numerous in the medulla. Consequently, the stratified vegetative structure seen in higher-order branches was less distinct. The stratified structure may be lost entirely in the basal parts of main axes, consisting instead of homogenously distributed pigmented cortical cells in a network of transversely and longitudinally oriented rhizines.

None of the examined members of the Gelidiaceae, apart from *Ptilophora* and *Beckerella*, had the characteristic vegetative structure described above, as all lacked the inner cortical layer of large elliptical to spherical cells. A distinct rhizine band concentrated between the outer cortex and medulla and traversed by inner cortical cells was only observed in *Gelidium pteridifolium* R.E. Norris, Hommersand & Fredericq, *G. sesquipedale* (Clemente) Thuret and *G. asperum* (C. Agardh) Greville. All species except *G. sesquipedale* and *G. asperum* also had rhizines interspersed throughout the medulla. In *Pterocladia lucida* (R. Brown ex Turner) J. Agardh, *Acanthopeltis japonica* Okamura and *Gelidium vittatum* (Linnaeus) Kützing, rhizines were concentrated mostly in the medulla. The vegetative structure of *Pterocladia lucida*, *Gelidium coulteri* Harvey, *G. chilense* (Montagne) Santelices & Montalva, *G. pulchellum* (Turner) Kützing, *G. latifolium* Bornet *ex* Hauck, *G. caulacantheum* J. Agardh, *G. japonicum* (Harvey) Okamura and *G. vittatum* was not stratified into distinct tissue types. *Gelidium asperum* most closely approximated the vegetative structure of *Ptilophora* but had a homogenous mix of thick-walled elliptical medullary cells of large diameter and thick-walled cylindrical medullary cells that were much smaller in diameter. In longitudinal section, both types of cells were longitudinally elongated and roughly the same length. *Gelidium abbottiorum* R.E. Norris had a distinct rhizine band traversed by anticlinally arranged filaments of inner cortical cells, much like that seen in species of *Ptilophora* and *Beckerella*.

DISCUSSION

Surface proliferations were observed in all five species of *Ptilophora* and also four of the nine *Beckerella* species examined in this study. This character is therefore inadequate for distinguishing between *Ptilophora* and *Beckerella*. The morphology and density of surface proliferations varies among those species that posses them. *Ptilophora prolifera*, *P. spissa* and *P. diversifolia* produce a large number of surface proliferations but show variation in proliferation arrangement and morphology. Relatively few surface proliferations of similar morphology were found on specimens of *P. rhodoptera*, *Beckerella hildebrandtii* and *B. pinnatifida*. The two undescribed species included in this study were provisionally assigned to *Ptilophora* or *Beckerella* based on the presence or absence of surface proliferations. *Ptilophora sp.* produced numerous surface proliferations similar in morphology to those of *P. prolifera*, although only two surface proliferations were found on one of the eight *Beckerella sp.* specimens examined. Due to the length and single-layered outer cortex of the distally occurring surface proliferations of *B. scalaramosa*, these are interpreted as adventitious rhizoids like those produced on frond margins of *B. mediterranea*. The proliferations produced proximally on this species, also described in Kraft (1976), are more than likely rhizoidal primordia as they are only produced at the base of axes and are similar in morphology to the rhizoidal primordia observed on rhizoids of the same specimen. No consistent differences between *Ptilophora* and *Beckerella* were found in the external morphology or vegetative structure of surface proliferations, and the noted differences in surface proliferation morphology and arrangement are probably species specific. The observed within-species variation in surface proliferation characteristics may be the product of specimen age or environment, and indicates that morphological character states need to be assessed over a broad range of specimens.

Phylogenetic analyses of DNA sequence data from the chloroplast-encoded *rbc*L and nuclear-encoded LSU genes indicate that *Ptilophora* and *Beckerella* are not separate monophyletic groups. In all analyses *Beckerella* was paraphyletic with respect to *Ptilophora*, a relatively large penalty to parsimony being necessary to resolve *Ptilophora* and *Beckerella* as separate monophyletic clades. Based on these molecular data and the inconsistency found in the key morphological character distinguishing *Ptilophora* and *Beckerella*, their maintenance as separate genera is untenable.

Although *Ptilophora* and *Beckerella* were not resolved as separate monophyletic clades in molecular analyses, species of both genera are resolved together in a single monophyletic clade. A '*Ptilophora*' clade was also resolved in previous molecular analyses that included only two or three species referable to *Beckerella* (Freshwater *et al.* 1995; Freshwater & Bailey 1998; Thomas & Freshwater 2001). This study includes a more representative sampling of both *Ptilophora* and *Beckerella* species and resulted in a strongly supported *Ptilophora/Beckerella* clade in the *rbc*L tree and, depending upon the tree-building method, a moderately to strongly supported clade in the LSU tree.

Morphological comparisons of Gelidiales species show that only species of *Ptilophora* and *Beckerella* have a vegetative structure characterised by four distinct and concentric layers of homogeneous tissue types. The presence of an inner cortex of inflated cells was especially characteristic of both genera. Species differed predominantly in the thickness of the rhizine band and inner cortex and the size of the inner cortical cells. It was not determined whether these differences were species specific or indicative of different thallus ages or of environmental conditions experienced during growth. Vegetative structure instead of cystocarpic structure is the only known morphological synapomorphy for the species of *Ptilophora* and *Beckerella* at this stage.

The distinctive vegetative structure of *Ptilophora* and *Beckerella* is most clearly visible in mature regions where the thallus has a midrib or is thicker and less flattened, especially in *B. irregularis,* which lacks a midrib (Akatsuka & Masaki, 1983). It is suggested that transverse sections of second-order branches be made to confirm generic identification since the anatomy of the oldest parts of thalli are complicated by increased cortication.

The results of this study support the conclusion drawn by Norris (1987a) that *Ptilophora* and *Beckerella* are congeneric. The species of *Beckerella* are maintained in *Ptilophora*, and *Ptilophora* now contains 14 species (Table 2.2). Norris's (1987a) emendation of *Ptilophora* was concise, comprehensive and needs not be repeated here except to note an increase in the stated maximum size of thalli from 0.5 to 1 meter based on *P. subcostata* (Okamura 1909).

Table 2.2. List of species currently included in *Ptilophora*, with corresponding nomenclatural authorities.

Ptilophora biserrata (Børgesen) R.E. Norris 1987a: 256
Ptilophora diversifolia (Suhr) Papenfuss 1940: 214-216
Ptilophora hildebrandtii (Hauck) R.E. Norris, 1990: 133-134
Ptilophora irregularis (Akatsuka & Masaki) R.E. Norris 1987a: 256
Ptilophora mediterranea (H. Huvé) R.E. Norris 1987a: 258
Ptilophora pinnatifida J. Agardh 1885: 79
Ptilophora prolifera (Harvey) J.Agardh 1876: 555
Ptilophora pterocladioides Andriamampandry 1988: 244-247
Ptilophora rhodoptera R.E. Norris 1987a: 254
Ptilophora rumpii (Dickinson) R.E. Norris 1987a: 254-256
Ptilophora scalaramosa (Kraft) R.E. Norris 1987a: 258
Ptilophora spissa (Suhr) Kützing 1847: 25
Ptilophora subcostata (Okamura) R.E. Norris 1987a: 258
Ptilophora pectinata (A. & E.S. Gepp) R.E. Norris 1987a: 252 (including *Beckerella helenae* (Dickinson) Fan & Papenfuss, Norris 1992)

The recent merger of *Beckerella* with *Ptilophora* (Norris 1987a), *Yatabella* Okamura with *Acanthopeltis* Okamura in Yatabe (Shimada *et al.*, 1999) and the merger of *Onikusa* Akatsuka and *Suhria* J. Agardh in Endlicher with *Gelidium* (Chapter 1) leave eight recognised genera within the Gelidiales. Further study of *Acanthopeltis*, *Capreolia* Guiry & Womersley and *Porphyroglossum* Kützing are necessary to determine the status of these genera within a phylogeny-based natural classification system of the Gelidiales.

CHAPTER 3

TWO NEW SPECIES OF GELIDIALES (RHODOPHTA) FROM SOUTH AFRICA, *PTILOPHORA LELIAERTII* AND *PTILOPHORA COPPEJANSII*

INTRODUCTION

The concept of *Ptilophora* is clearly defined in Norris (1987a). In summary, *Ptilophora* is characterized by a large distichously branched frond (sometimes bearing minute surface proliferations). It usually has a distinct midrib in proximal parts, and is attached to the substratum by a fibrous, often robust, holdfast. Plants have a characteristic four-layered vegetative structure including a well defined, large-celled inner cortex of anticlinally arranged, round to elliptical cells in transverse section. *Ptilophora* species generally lack single discrete defining characters and are instead defined by a combination of external morphological characters such as frond width, pinnule shape, the distinctiveness of the midrib, branching pattern, maximum branch order, and the distance of ramuli from the axis. Although cell dimensions and other details of the vegetative structure have often been provided in the species descriptions, they have not been used as diagnostic characters for any of the species.

The recent taxonomic research on *Ptilophora* (Norris 1987a, 1990b, 1992b) focused on the species occurring along the coast of the KwaZulu-Natal Province (KZN) of South Africa. Consequently, the species that occur in this region are well described and most have been repeatedly collected. This previous research greatly facilitated in the identification of two new species endemic to this area, namely *Ptilophora coppejansii* Tronchin and *Ptilophora leliaertii* Tronchin, described here from Protea Banks Reef situated off the coast of southern KZN (Fig. 1). The species delimitations have been based on specific attributes of external branch morphology and strong molecular support from DNA sequence data. This is the first time that species in the genus *Ptilophora* have been proposed with the support of molecular data. *Ptilophora leliaertii* has previously been misidentified as *Ptilophora pinnatifida* (Freshwater *et al.* 1995, Bailey & Freshwater 1997, Freshwater & Bailey 1998, Freshwater *et al.* 1999, Thomas & Freshwater 2001, Rico *et al.* 2002) and referred to as *Beckerella sp.* in chapter 2 where *Ptilophora coppejansii* was also referred to as *Ptilophora sp.*

Ptilophora helenae (Dickinson) Norris, a pectinate species reported only from Mission Rocks and beach-cast drift at Richards Bay along the northern KZN coastline, was placed in synonymy with *Ptilophora pectinata* (A. Gepp & E. Gepp)

Norris from Australia by Norris (1992b) due to a lack of reliable differences between the species. *Ptilophora helenae* is resurrected in this chapter based on molecular and morphological data.

MATERIALS AND METHODS

Morphological observations

Specimens of the new species were obtained during dives on Protea Banks Reef during August of 1999, January and June of 2000 and February of 2001. Additional specimens were examined from the herbaria of the University of Natal (NU) and University of Cape Town (BOL). These specimens were compared to specimens of all currently accepted *Ptilophora* species, either collected on the same field trips (including pressed, wet preserved or silica gel dried material) or from national and international herbaria (including nine type specimens). Observations of whole specimens were made using a Wild M10 stereo dissecting photomicroscope and photos were taken with an Olympus Camedia digital camera. Hand sections were cut from pressed or silica dried specimens, stained with 1% aniline blue stain and preserved in a 50% Karo^{TM} solution. Photos of sections were taken with an Olympus DP50 digital camera mounted on a Leitz Diaplan compound photomicroscope. Using image analysis software (UTHSCSA Image tool v. 3), morphometric data were obtained from various specimens of *P. leliaertii* and *P. 'pectinata'* from South Africa and Australia, including the type specimens of both species.

Molecular analyses

Specimens used for *rbc*L and LSU gene sequence analysis were collected from field sites and dried using silica gel desiccant. Samples were also taken from herbarium specimens including type material. For the protocol used for total genomic DNA extraction, *rbc*L and LSU gene amplification and sequencing, sequence alignment and the determination of data characteristics, refer to the materials and method section of chapter 2. Refer to the CD-ROM attached for *rbc*L and LSU gene data files including all the sequences analysed in this thesis.

Distance, maximum parsimony and maximum likelihood analyses were performed on *rbc*L and LSU data files using PAUP (v. 4.0b10, Swofford 2001). Eighteen sequences (including 5 previously published) were included in analyses of the *rbc*L sequence data, and 13 sequences (including 9 previously published) in analyses of the partial LSU sequence data. Due to missing data at the 5' ends of *rbc*L and LSU sequences, the first 67 sites of the 1467 bp *rbc*L gene and first 7 sites of the 1150 bp LSU alignment were excluded from the analysis such that at least 50% of the sequences had complete data at the 5' end.

Distance trees were generated using neighbour-joining tree building with Tamura-Nei distances. The Tamura-Nei correction was used since both data sets had an unequal frequency of bases and a purine-purine or pyrimidine-pyrimidine transition bias. Distance bootstrap analyses consisted of 2000 replicates of neighbour-joining tree building with Tamura Nei distances. Maximum parsimony analyses of the *rbc*L sequence data consisted of a heuristic search performing 1000 random sequence additions (holding 50 trees at each step during stepwise addition) using the tree-bisection-reconnection (TBR) branch-swapping algorithm with MULTREES and STEEPEST DESCENT options in effect. Decay indices were determined based on strict consensus analyses of cladograms found by relaxing parsimony sequentially, one step at a time, up to five steps (Freshwater and Rueness, 1994). Maximum parsimony analyses of the LSU sequence data were performed with the branch-and-bound search algorithm, simple sequence addition and the MULTREES option in effect. Maximum parsimony bootstrap analyses of the *rbc*L data consisted of 1000 replications of heuristic searches with simple sequence addition, MULTREES, STEEPEST DESCENT, and TBR. For the LSU sequence data, 1000 replications of branch-and-bound searches with simple sequence addition and MULTREES were used. Optimality criteria for maximum likelihood analyses were determined with Modeltest v. 3.06 (Posada & Crandal 1998). Maximum likelihood analyses of *rbc*L and LSU sequence data consisted of 10 separate searches of random sequence additions with TBR branch swapping and MULTREES. Maximum likelihood bootstrap analyses consisted of 300 (*rbc*L) or 500 (LSU) replications of one random sequence addition, with MULTREES and TBR branch swapping. Quartet-puzzling analyses of 1000 puzzlings were performed on the LSU sequence data using the same model parameters as in likelihood searches.

RESULTS

Ptilophora leliaertii Tronchin nom. prov.

Description

Plants up to 35 cm tall arising from a fibrous holdfast of robust, terete parts. Decumbent, branched rhizoids may develop proximally on the main axis and lower branches. Rhizoids cylindrical producing brush-like anchoring haptera at their ends. Lower axis cylindrical (ca. 2 mm in diameter) becoming narrowly alate in lower branches and flattened and concave in the pinnate reaches with midrib less distinct. Up to six orders of regular distichous, compound pinnate, opposite to alternate branching. Flattened branches mostly 1.5-2 mm broad and fairly uniformly spaced, 1-2 mm apart. Branch apices rounded or tapered to a sharp point particularly in young or primordial pinnae. Branches concave, and except for ultimate pinnae, are generally broader (up to 30%) than their bearing branch and roughly as wide at their base as the bearing branch. Surface proliferations are uncommon. Plants have a four-layered vegetative structure. Outer cortical cells round to quadrangular, surrounding a distinct band of thick-walled rhizines traversed by anticlinal rows of outer cortical cells. Inner cortex 3-4 layers thick, cells periclinally arranged, round to elliptical. Medulla filamentous. Fertile structures borne terminally or subterminally on lateral branchlets. Cystocarps are bilocular and usually have a distal protuberance. Spermatangial and tetrasporangial sori elliptical, borne on short, slightly swollen branchlets. Tetraspores cruciate.

Etymology: This species is named after Frederik Leliaert of the Phycology Research Group at Ghent University, for having collected the first attached specimen of the species, all previously collected specimens having come from beach-cast drift.
Holotype: 34657 (BOL), ET16 (Fig. 3.1), collected by E.M. Tronchin on 4 February 2001
Type locality: Protea Banks Reef (7.5 km offshore from Shelly Beach), KwaZulu-Natal Province, South Africa.

Distribution: Known only from the type locality and drift at Uvongo beach (5 km north of Shelly beach).

Specimens examined: Protea Banks (KZN), Southern Pinnacle, -34 m; Leliaert *et al.*; 20 viii 1999; KZN 881 (GENT, BOL). Protea Banks, Southern Pinnacle, -37 m; Tronchin; 4 ii 2001; 99068 (BOL). Protea Banks, Northern Pinnacle, -35 m; Anderson and Bolton; 29 vi 2000; KZN 1857 (GENT). Location unknown; Simons; 1966; 109599 (BOL). Protea Banks, Southern Pinnacle, -34m; Leliaert; 20 viii 1999; 512 (BOL) - wet preserved. Palm Beach (KZN) - drift; Hommersand; 23 vii 1993; s.n. Uvongo Rocks (drift); Pocock; 1 xi 1951; 10090 (GRA).

Ecology

This species was collected from Protea Banks Reef between -34 m and -37 m attached to exposed flat reef surfaces in mixed algal beds at Northern Pinnacle (S 30.49.616 E 30.29.418); and Southern Pinnacle (S 30.50.319 E 30.28.885), and turf-covered reef outcrops at Southern Pinnacle. Algae form the aspect-dominating group of organisms at Protea Banks, reaching an unusual biomass for subtropical regions. The meadows of *Ptilophora* spp. (*P. pinnatifida*, *P. leliaertii* and *P. rhodoptera*) are intermixed with *Codiophyllum natalense* J. Gray, *Thamnoclonium dichotomum* (J. Agardh) J. Agardh, *Cryptonemia natalensis* (J. Agardh) Chiang, *Zonaria subarticulata* (Lamouroux) Papenfuss and *Dictyota liturata* J. Agardh and offer a spectacular sight. The rocky reef is often partially covered in sand and other particulate matter originating predominantly from the numerous estuaries along the southern KZN coastline. Turbid water frequently drifts over the reef due to a strong prevailing current.

Habit

Plants can attain 35 cm in height and are attached by a fibrous holdfast of robust, terete rhizoids. Branched rhizoids may issue from the lower axis and less commonly from lower branches (Fig. 3.2). Where the rhizoids contact the substratum they produce brush-like anchoring haptera composed of elongate aseptate cells (Fig. 3.3). With increasing distance from the holdfast there is a transition from the rhizoids described above to short (< 0.5 cm), simple and apically-directed adventitious

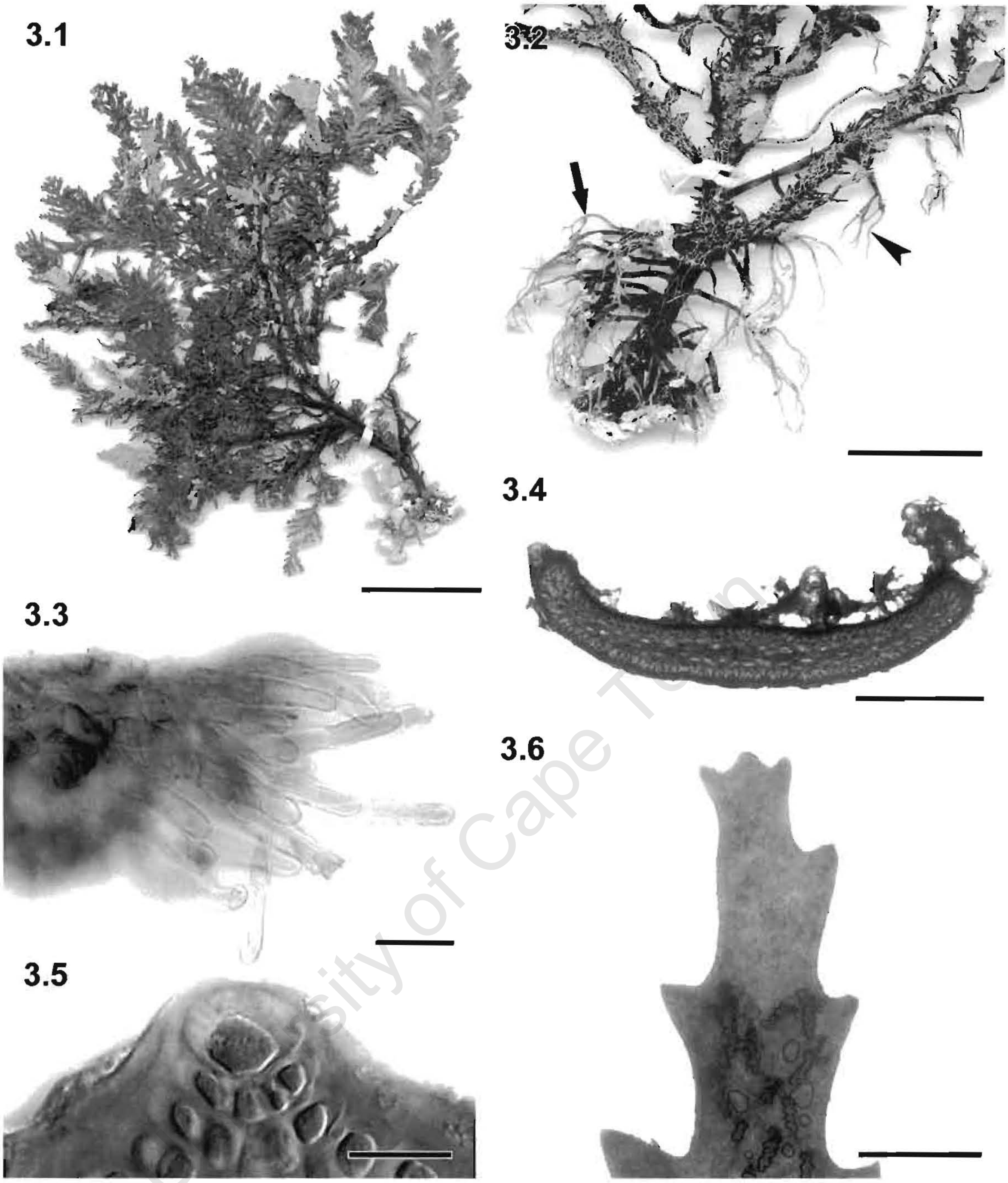

Fig. 3.1-3.6. *Ptilophora leliaertii*. Fig. 3.1. Holotype specimen covered in places by sponge and crustose coralline epiphytes. Scale = 4 cm. Fig. 3.2. Holdfast with rhizoids developing from the lower axis (arrow) as well as from lower branches (arrowhead). Scale = 1 cm. Fig. 3.3. Brush-type anchoring hapteron of elongate aseptate cells developing from the end of a cylindrical rhizoid. Scale = 50 µm. Fig. 3.4. Transverse section of a distal concave branch. A hydroid and sponge epiphyte are attached predominantly to the concave surface of the frond. The sponge spicule skeleton was poorly preserved during the sectioning procedure. Scale = 250 µm. Fig. 3.5. Surface view of protrusive apical cell. Scale = 10 µm. Fig. 3.6. Surface view of apical tip of pinnate branch. Scale = 1 mm.

branchlets in lower parts of the thallus arising on the thallus surface. Such surface proliferations are normally scarce, especially in the pinnate regions of the thallus.

The frond has a cylindrical lower axis (ca. 2 mm in diameter) becoming narrowly alate with a subcylindrical midrib in lower order branches. Branches are flattened and concave in the pinnate regions (Fig. 3.4) where the midrib is less distinct. The basal parts of the axis and primary branches are less branched. There are up to six orders of regular distichous, opposite to alternate branches. Flattened branches are mostly 1.5-2 (-2.5) mm broad and fairly uniformly spaced, generally 1-2 (-2.5) mm apart. Branch apices can taper to a sharp point with a protuberant apical cell (Fig. 3.5) particularly in young or primordial pinnae, and become rounded with age. Apices of indeterminate branches are often tridentate (Fig. 3.6).

In flattened regions, branches, except ultimate pinnae, are generally broader (up to 30%) than their bearing branch, though sometimes uniform in width, and are roughly as wide at their base as the bearing branch (Fig. 3.7). The branch usually bends adaxially after its junction with the bearing branch and then broadens slightly. Margins of concave branches start to in-roll at this basal branch bend. Pinnae and their bearing branch are decurrently united with rounded axils.

A specimen found at NU (Fig. 3.8) is thought to represent an aberrant morphology of this species. The specimen bears many branched, lateral proliferations that may reticulate.

Vegetative structure

Plants have a four-layered vegetative structure (Fig. 3.9). In transverse sections of second order branches the outer cortex is composed of a single layer of round to quadrangular cells, (5-) 7-8.5 (-10) µm in length, 5-8 µm wide, with a length width ration of 1-2. Rhizines are 3-5 µm in diameter and form a distinct band traversed by anticlinal rows of pigmented outer cortical cell. These cells are angular, and more elongated (length/width ratio is greater than 2). The inner cortex consists of 3-4 layers of periclinally arranged round to elliptical cells in transverse section, (27-) 29-44 (-51) µm in length, (15-) 20-28 (-37) µm wide, with a length/width ratio of 0.8-2. The

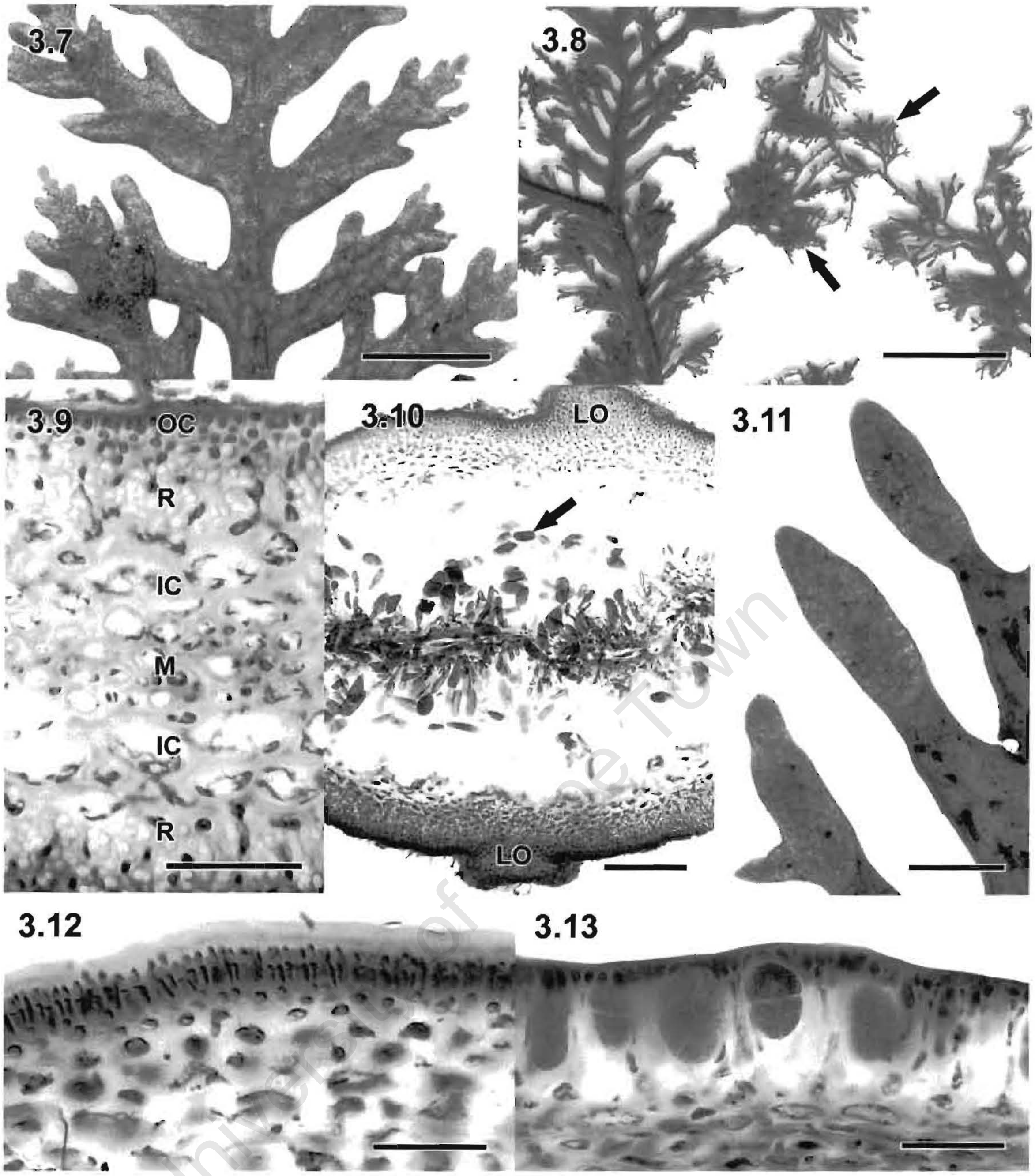

Fig. 3.7-3.13. *Ptilophora leliaertii.* Fig. 3.7. View of convex surface of compound branch. Scale = 3 mm. Fig. 3.8. An aberrant morphology of this species where finely branched, lateral proliferations (arrow) are produced. Scale = 1 cm. Fig. 3.9. Transverse section of second order branch. Outer cortex (OC); rhizine band (R); inner cortex (IC); medulla (M). Scale = 50 µm. Fig. 3.10. Transverse section of bilocular cystocarp with mature carposporangia (arrow) and lipped ostioles (LO). Scale = 100 µm. Fig. 3.11. Surface view of three pinnae with lightly pigmented terminal tetrasporangial sori. The sorus of one of the pinnae extends into a lateral pinnule. Scale = 1 mm. Fig. 3.12. Transverse section of fertile region of pinnule with an outer cortex of spermatangial mother cells with transverse division lines. Scale = 25 µm. Fig. 3.13. Transverse section of tetrasporangial branchlet with mature, cruciately divided tetrasporangium. Scale = 50 µm.

medulla consists of thick-walled filaments that are round in transverse section and 7-12μm in diameter, and is devoid of rhizines. Larger cells can sometimes occur in the medulla, approximating the appearance of small round inner cortical cells in transverse section. In higher order branches the medulla and midrib becomes less distinct. In proximal parts the stratified vegetative structure becomes obscured by increased cortication and rhizine concentration.

Reproduction

Reproductive structures are borne apically or subapically on lateral branchlets. Cystocarps are borne either on short branchlets or terminally on narrow ligules (up to 5 mm in length) usually with a distal protuberance. The cystocarps are bilocular, locules being elliptical in surface view, with one centrally placed ostiole that may have a peristome (Fig. 3.10) or may be depressed.

Spermatangia are borne on short, slightly lobed branchlets. Spermatangial mother cells form a continuous single-layered outer cortex easily recognized by their elongate shape, cutting off spermatangia by transverse division. (Fig. 3.12). Spermatangial sori develop on both surfaces of the pinnae and are surrounded by a sterile margin which is too narrow to be noticed in surface view.

Fertile tissue on tetrasporangial branchlets is clearly noticeable by its lighter pigmentation. The elliptical tetrasporangial sori occur on both surfaces of terminal branch swellings which can sometimes be continuous with the sori of lateral pinnae (Fig. 3.11). Sori are usually surrounded by a very thin sterile margin but may extend all the way around the flattened pinnule. The darkly pigmented tetraspores can usually be seen in surface view. Tetrasporangia are cruciately divided and when viewed in transverse sections are up to 58 μm long and up to 40 μm wide, with a length/width ratio of 1.2-2.4 (Fig. 3.13).

Epiphytes

Acrosorium acrospermum (J. Agardh) Kylin and a diversity of sponges, crustose corallines and hydroids are common epiphytes on *P. leliaertii*. Sponges and hydroids

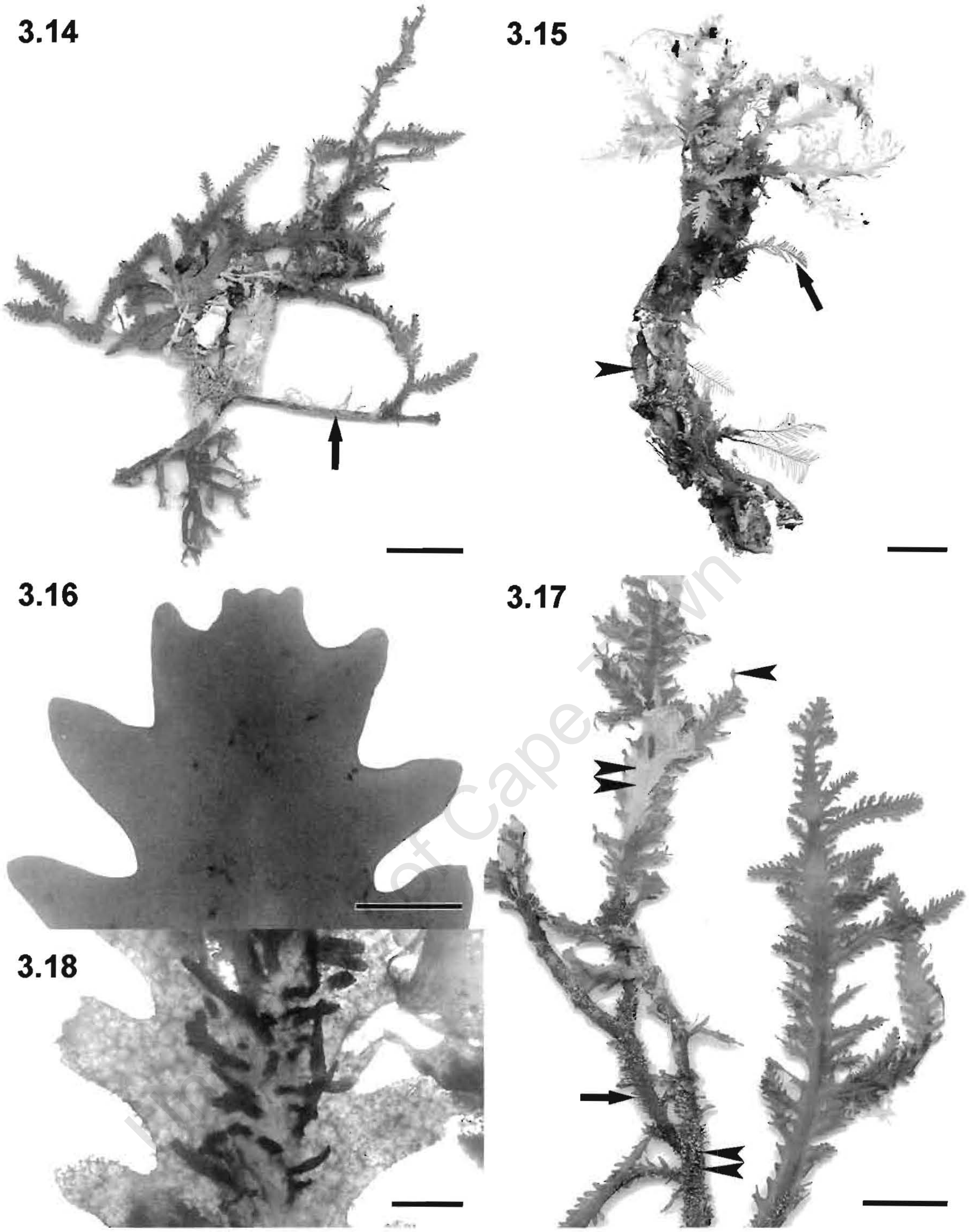

Fig. 3.14-3.18. *Ptilophora coppejansii*. Fig. 3.14. One of two fragments of the holotype of *Ptilophora coppejansii*. The axis and major branches are covered in a variety of epiphytes. Lower branches may become bare with age (arrow). Scale = 2 cm. Fig. 3.15. An aberrant morph with an axis (arrowhead) that is 6 mm in diameter. The axis is also covered in epiphytic sponge and a hydroid (arrow). Scale = 1 cm. Fig. 3.16. Surface view of a branch apex. Scale = 1 mm. Fig. 3.17. Distal pinnate branches. An aberrant morphology, illustrated here, that may be encountered is the production of closely spaced, fine and lanceolate (sometimes pinnate) proliferations (arrow) on blade margins. The regions of the thallus with this morphology are covered in sponge epiphyte. Sponge epiphyte also occurs on parts of the frond with the normally encountered branch morphology (arrowheads). Cystocarpic branchlets (arrowhead) are born on several pinnae. Scale = 1 cm. Fig. 3.18. Simple surface proliferations (arrow) arising from the midrib of a second order branch. The sponge encrustation covering the branch was removed and only a thin film remains. Scale = 1 cm.

Type locality: Protea Banks Reef (7.5 km offshore from Shelly Beach), KwaZulu-Natal Province, South Africa.
Distribution: Known only from the type locality.
Specimens examined: Protea Banks (KZN), Northern Pinnacle, -35 m; Anderson & Bolton; 29 vi 2000; KZN 1858 (GENT). Protea Banks, Salmon Bank, -27 m; Tronchin; 6 ii 2001; KZN 1992 (GENT) – wet preserved. Protea Banks, Southern Pinnacle, -34 m; Leliaert; 20 viii 1999; 511 (BOL) – wet preserved. Protea Banks, Southern Pinnacle, -37 m; Freshwater; 4 ii 2001; 99070 (BOL).

Ecology

This species was found on Protea Banks Reef in mixed algal beds at Northern Pinnacle (S 30.49.616 E 30.29.418); Southern Pinnacle (S 30.50.319 E 30.28.885) and Salmon Banks (S 30.49.220 E 30.25.316) at depths between -27 and -37 m. It occurs on open reef flats that are usually exposed to a strong prevailing current and are covered predominantly by sand, particulate matter and turf algae.

Habit

Plants have been recorded to grow up to 23 cm in height. They have a branching, fibrous holdfast which is robust and rigid and consists of terete rhizoids, up to 3 mm in diameter (Fig. 3.14), which terminate in brush-like attachment haptera composed of aseptate, elongate cells. The lower axis is cylindrical, rigid, usually 2-3mm wide, though axes as wide as 6 mm can be encountered (Fig. 3.15). Plants have up to four orders of pinnate branching. Branching in the lower regions of the thallus is irregular and sparse. In the central and distal regions branching is regular, more dense, and opposite to alternate. In large plants, certain lower branches may lack alae and lateral branches (Fig.14). Branches of all orders, except ultimate pinnae, have a distinct midrib to just short of the apex and are pinnatifid. Branches are generally 2-3 mm broad, tapering or broadening slightly in sections, and are never broader than their bearing branch. Pinnae are narrow (< 1 mm), short (up to 3 mm long) and closely space (<1.3 mm apart) (Fig. 3.14, 3.17). Pinnae are generally simple with a slight geniculate bend and taper rapidly to an acute or rounded apex. The margins of the pinnae may be serrate due to developing branch primordia. The apices of pinnae-bearing axes tend to be tridentate, the middle tooth being broadest, beset on either side by narrower teeth (Fig. 3.16). Surface proliferations which are simple and ligulate to three times pinnate, are regularly produced from the subapical parts downwards (Fig. 3.18).

This species commonly develops aberrant morphologies. Some thalli may produce numerous fine, lanceolate (sometimes pinnate) proliferations in close proximity on the margins (Fig. 3.17). These regions may be covered by sponge epiphytes. Some aberrant growth forms may produce more than four orders of branching. Major branching can be very irregular and may be produced in three dimensions. In some specimens, totally enveloped by sponge (Fig. 3.19), the morphology is particularly aberrant: branches may be very closely spaced or covered in either pinnate surface proliferations, or tufts of digitate, simple surface proliferations borne from the same point on the branch surface. Reticulating lateral proliferations are also produced. Blades may be finely branched, subcylindrical rather than flattened, and can have abnormally broad, contoured and abruptly terminated apices (Fig. 3.20).

Vegetative structure

Plants have a four-layered vegetative structure (Fig. 3.21). In transverse sections of second-order branches, the outer cortex consists of heavily pigmented, quadrangular cells. The cells are 8-10 µm in length, 5-6 µm wide, with a length/width ratio of 1.3-2. A distinct rhizine band separates the outer cortex from the large-celled inner cortex and is traversed by angular, pigmented cortical cell filaments that are anticlinally arranged. The rhizines are usually 4-5 µm in diameter. The inner cortex consists of 2-3 layers of periclinally arranged, round or elliptical cells in transverse section. Cells are (20-) 25-40 µm in length, 14-30 µm wide, with a length/width ratio of 1.2-1.9. Medullary filaments are 6-10 µm in diameter with larger cells (15-20 µm) interspersed. Rhizines may occur towards the periphery of the medulla.

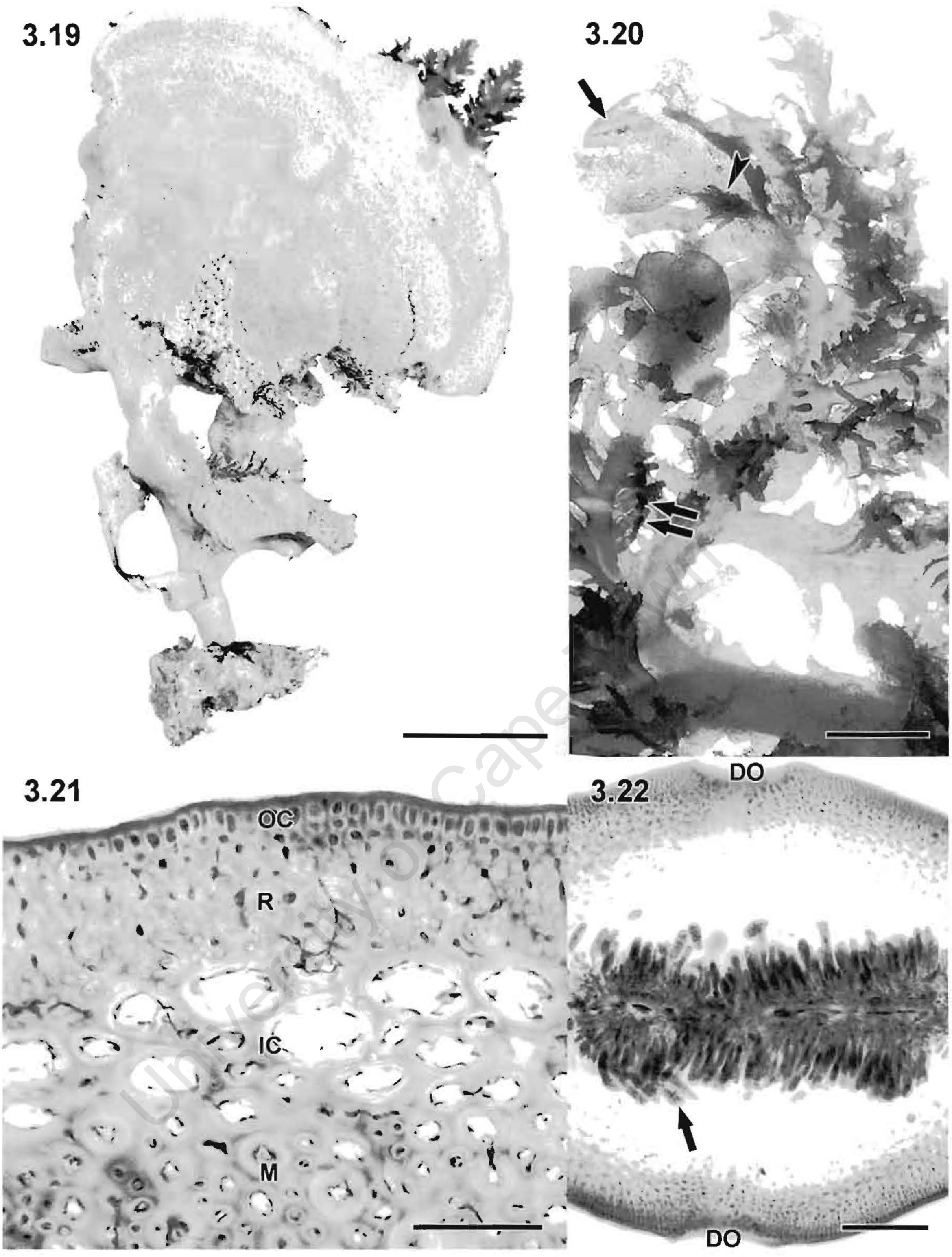

Fig. 3.19-3.22. *Ptilophora coppejansii*. Fig. 3.19. A plant that is completely enveloped by an epiphytic sponge, except for the holdfast and a few distal branch tips. Scale = 2 cm. Fig. 3.20. A lateral branch of a totally sponge-encrusted plant with the sponge encrustation removed. The branch morphology is aberrant, characterised by abnormal branch apices (single arrow), extensive surface proliferations that are sometimes in tufts (arrowhead), and fine, closely spaced lateral branching (double arrows). Scale = 3 mm. Fig. 3.21. Transverse section of a second order branch. Outer cortex (OC); inner cortex IC; rhizine band (R); medulla (M). Scale = 50 µm. Fig. 3.22. Transverse section of a bilocular cystocarp with elongate carposporangia (arrow) produced from the placental mass. Each locule has one depressed ostiole (DO). Scale = 100 µm.

Reproduction

Fertile structures are borne terminally or subterminally on branches. Cystocarpic branchlets are acuminate proximally and have a distal protuberance (Fig. 3.17). Cystocarps are bilocular and have one depressed ostiole per locule (Fig. 3.22). Male or tetrasporophytic plants have not been found.

Epiphytes

Ptilophora coppejansii is commonly associated with sponge and hydroid epiphytes. In some cases of sponge epiphytism, the association can be so extensive that the seaweed may easily be mistaken for a sponge.

***rbc*L Analysis**

The analysed *rbc*L data set included 18 taxa and 1400 nucleotide sites. 270 Sites (19.3%) were variable of which 149 (10.6%) were parsimony-informative. The data set had an unequal frequency of bases (A=30.6%; C=16.5%; G=21.3%; T=31.1%) and a transition:transversion ratio of 4.01 with a 60.4% bias towards pyrimidine-pyrimidine transitions. A maximum likelihood analysis of these data produced a tree with a likelihood value of -4219.99 (Fig. 3.23). Its topology was nearly identical to one of two most parsimonious trees of 424 steps generated by the parsimony analysis, differing only in the placement of *Ptilophora pterocladioides* Andriamampandry which was the most basal taxon in the maximum parsimony trees. The topology of the maximum likelihood tree differed to that of the neighbour-joining tree in the arrangement of the basal taxa *P. pterocladioides*, *Ptilophora mediterranea* (H. Huvé) Norris and *P. pectinata* from Australia. Neighbour joining and maximum parsimony trees were nearly identical, differing in the arrangement of basal lineages. There was weak to no support for the arrangement of the basal lineages with all analysis methods employed.

The analysed LSU data set included 13 taxa and 1143 sites. 56 Sites (4.7%) were variable of which 15 (1.3%) were parsimony-informative. The data set had an unequal frequency of bases (A=23.4%; C=22.1%; G=31.2%; T=23.3%), a

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All *rbc*L trees generated by distance, maximum parsimony and maximum likelihood methods included a clade containing *P. coppejansii, P. diversifolia, P. leliaertii, P.' pectinata'* from South Africa and *Ptilophora rhodoptera* Norris. The sister relationships within this clade were always identical. Within this clade, three separate plants of *P. coppejansii* collected from different locations on Protea Banks Reef (two from Southern Pinnacle and one from Salmon Banks) had identical *rbc*L sequences and formed a strongly supported species clade. *Ptilophora diversifolia* from Protea Banks was resolved as a sister taxon to this species with *P. rhodoptera* sister to this clade. *Ptilophora coppejansii* and *P. rhodoptera* had identical LSU sequences and both species were resolved in a well supported clade with *P. diversifolia*.

Two specimens of *P. leliaertii*, one from Protea Banks and one from drift washed up at Palm Beach (ca. 20 km further south along the coastline) had very similar *rbc*L sequences (0.3% sequence divergence between the two) which differed by at least 2.3% from other *Ptilophora* species from the same locations. In the *rbc*L phylogeny, *P. 'pectinata'* from Mission Rocks (South Africa) differed by 0.4% sequence divergence from *P. leliaertii* from Protea Banks. These two species had identical LSU gene sequences.

In the analyses of both *rbc*L and LSU data sets, *P. 'pectinata'* was molecularly very different from *P. pectinata* from Australia, the former being consistently resolved in a strongly supported clade of South African taxa and the latter being consistently resolved as a basal lineage. Despite superficial morphological similarities (Table 3.1) between South African and Australian plants, a morphometric investigation of two

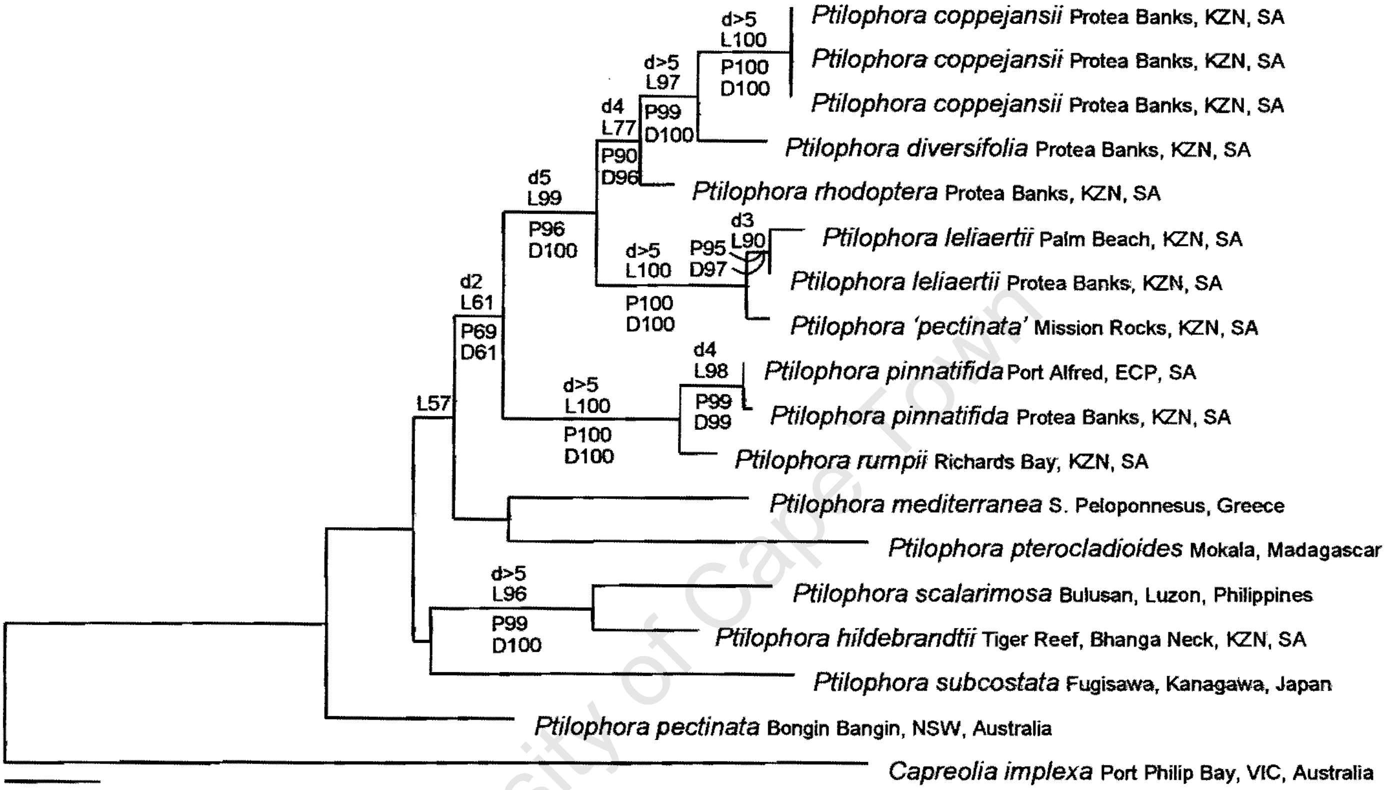


Fig. 3.23. Maximum likelihood tree constructed from 18 aligned *rbc*L sequences from *Ptilophora* species using a general time reversible model (r-matrix: A-C=1.34, A-G=1.27, A-T=0.43, C-G=1.4, C-T=2.29, G-T=1; a=0.1974). Branch lengths are proportional to the number of the expected nucleotide substitutions. Estimates of branch support provided include decay indices as well as bootstrap support (%) when ≥ 50 (L = maximum likelihood; P = parsimony; D = distance). Eastern Cape Province (ECP); KwaZulu-Natal Province (KZN); New South Wales (NSW); South Africa (SA); Victoria (VIC). The sequences for *P. mediterranea* and *P. pterocladioides* were obtained from isotype and holotype specimens respectively. Scale = 0.01 substitution per site.

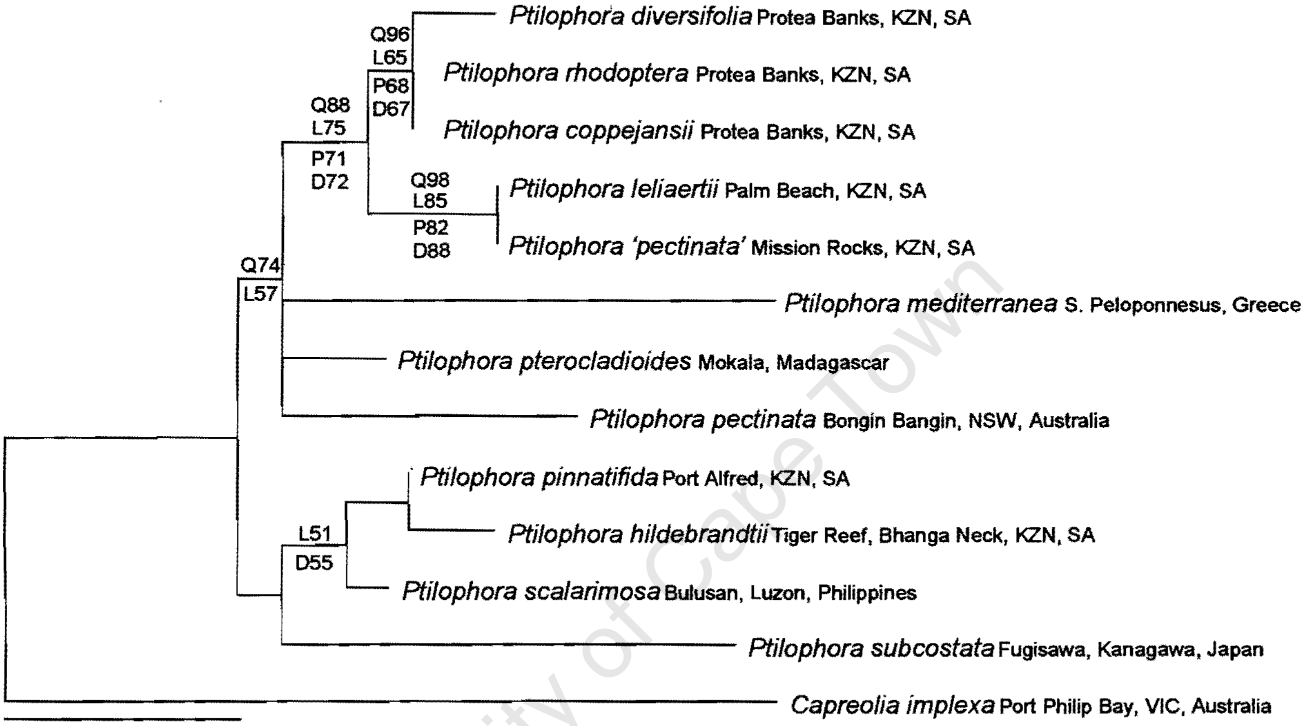


Fig. 3.24. Maximum likelihood tree constructed from 14 aligned LSU sequences from *Ptilophora* species using the Hasegawa-Kishino-Yano (1985) model and a ti:tv = 1.853. Branch lengths are proportional to the number of the expected nucleotide substitutions. Bootstrap support (%) and quartet puzzling reliability values are given for branches when ≥ 50 (Q = quartet puzzling; L = maximum likelihood; P = parsimony; D = distance). KwaZulu-Natal Province (KZN); New South Wales (NSW); South Africa (SA); Victoria (VIC). The sequences for *P. mediterranea* and *P. pterocladioides* were obtained from the isotype and holotype specimens respectively. Scale = 0.005 substitution per site.

morphological characters revealed that these specimens differed consistently in the distance on primary branches from the junction with the axis to the point at which ramuli commence. This distance is larger in plants from Australia (Fig. 3.25).

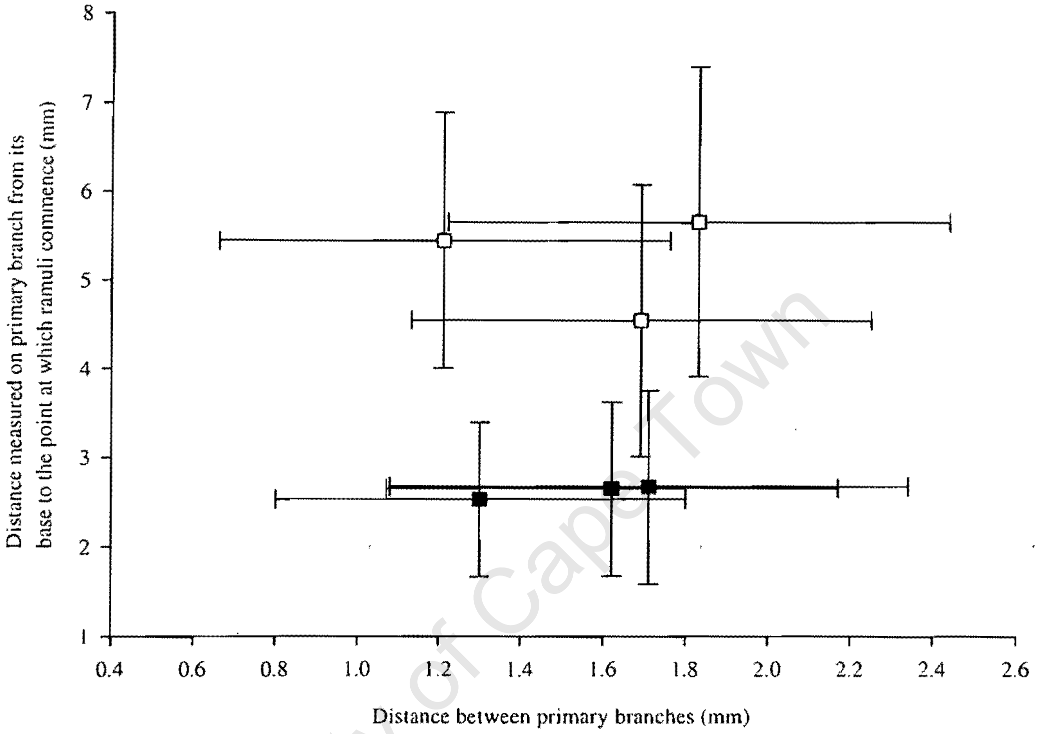


Fig. 3.25. The means and standard deviations of two dimensions measured from specimens of *Ptilophora pectinata* (□) and *Ptilophora helenae* (■).

DISCUSSION

The basal tree topology varied between all trees generated depending on the sequence data or analysis method used. This would seem to indicate that the basal lineages have diverged from each other over a short period of time evolutionarily, and neither *rbc*L nor LSU gene sequences are able to clearly resolve them.

All *rbc*L analyses provide strong support for the recognition of *P. coppejansii* and *P. leliaertii* as distinct species. LSU analyses did not support this, but LSU sequences are more conserved than *rbc*L sequences among Gelidiales taxa and may not vary between closely related species (Freshwater & Bailey 1998, Freshwater *et al.* 1999). The difference between *rbc*L sequences of *P. leliaertii* and *P. 'pectinata'* from South Africa falls within the ranges of intraspecific sequence variability reported for various other species in the Gelidiales (Freshwater and Rueness, 1994). However, species have been recognized previously despite their *rbc*L sequences being ≤0.3% divergent from their sister taxa (Shimada *et al.* 1999, 2000a). Sequence divergence can be useful as a guide in the delimitation of species but is confounded by variation in the pace of molecular evolution (mutation occurrence and fixation), and the fact that morphological and protein characters may evolve independently, the pace of which may differ between recently diverged species (Ferguson, 1980). It is possible that there has not been a long enough period of separation for differences in fixed mutations to have built up in the *rbc*L gene loci of *P. leliaertii* and *P. 'pectinata'*, resulting in such similar sequences. Nonetheless, these two species are morphologically distinct. The difference lies particularly in the branching pattern: *P. 'pectinata'* produces procurrent axes and branches with rostrate to acuminate pinnae, whereas *P. leliaertii* is compound pinnate throughout, producing up to two more branch orders than *P. 'pectinata'* (Table 3.1), pinnae are linear, and normally have obtuse apices. Furthermore, the distributions of these two species are likely to be considerably disjunct. *Ptilophora 'pectinata'* has only been collected from Mission Rocks and drift at Richards Bay (just south of Mission Rocks), Drift at Richard's Bay is likely to have originated from a locality either further north due to the prevailing Agulhas current that runs in a southwesterly direction along this coast, or just to the south of it due to counter currents produced by gyres. Richards Bay is ca. 315 km north of the known distribution of *P. leliaertii*. It is felt, therefore, that there is enough evidence to recognise *P. leliaertii* as a distinct species.

P. leliaertii is distinguished by the consistently concave nature of the blade and indistinct midrib throughout the flattened, compound pinnate parts. Concave branches are sometimes formed in *P. prolifera* and *P. coppejansii*, though in the case of the latter the alae often emerge from the midrib in a 'v-shape'. In both species, concave branches are only produced in parts of the thallus with pinnules almost never concave. Fronds of *P. leliaertii* are clearly differentiable from those of *P. prolifera* by being narrower, up to 2.5 mm as opposed to 6 mm wide, and by the very infrequent production of surface proliferations compared to the normal abundance of these structures in *P. prolifera*. Furthermore, in flattened regions of *P. prolifera* fronds, branches are always narrower than the bearing branch, whereas in *P. leliaertii* they can be up to 30% broader.

Ptilophora coppejansii is distinguished by long major branches and sparse, irregularly arranged indeterminate laterals in distal parts, with a clearly visible midrib beset on either side by a thin, flexuous ala, regularly producing small (up to 3 mm long), closely spaced (up to 1.3 mm apart) pinnae. The species that most closely resemble it are *P. diversifolia* and *P. rhodoptera*. These two species also have broad alae, distinct midribs and the same underlying branching pattern of elongate major branches producing sparse indeterminate laterals, but are not pinnate (Table 3.1).

Ptilophora 'pectinata' from South Africa and *P. pectinata* from Australia are molecularly very distinct, but morphologically practically identical (Table 3.1). Plants from these two highly disjunct geographical regions share the same diagnostic characters of rostrate to acuminate determinate laterals and procurrent axes and major branches. Vegetative structure, branching pattern, apical morphology, and thallus proportions such as branch width (Table 3.1) and between branch distances (Fig. 3.25), have not provided ways to distinguish between them. However, plants differ consistently in the average distance from the base of a primary branch to the point at which ramuli commence on that branch. There is little overlap in the standard deviations about the means for these two species (Fig. 3.25). Due to the very disjunct geographical ranges of South African and Australian plants, the aforementioned character difference, and the strong molecular support for two discrete taxa, plants from South Africa are considered to represent a separate species from those in Australia. The previous name applied to the South African plants, *Ptilophora helenae*, is hereby resurrected. *Ptilophora helenae* is a species endemic to the northern KZN coastline.

Table 3.1. Comparison of morphological characters of six species of *Ptilophora*.

	Ptilophora diversifolia	***Ptilophora rhodoptera***	***Ptilophora coppejansii***	***Ptilophora leliaertii***	***Ptilophora helenae***	***Ptilophora pectinata***
Maximum plant height (cm)	30	35	23	35	40	30
Proximal axis diameter (mm)	2.5 (-6)	3.5	3 (-6)	2	2	2 (-4)
Midrib distinct to naked eye in flattened blades	✓	✓	✓	x	x	x
Maximum branch order	4	3	4	6	4	4
Branch width (mm)	2-5	3-10	0.2-3	0.5-2.5	0.36-1.45	0.2-1.2
Branches always narrower than bearing branch	x	x	✓	x	✓	✓
Maximum distance between pinnae (mm)	not pinnate	not pinnate	1.3	2.5	2.8	3
Distal blades concave/undulate	undulate	undulate	neither	concave	neither	neither
Branch margins	undulate to crenate to eroded	blunt or mucronate serrations	entire	entire	entire	entire
Shape of ultimate pinnae	not pinnate	not pinnate	linear with rounded/acute tips	linear with obtuse/acute tip	rostrate to acuminate	rostrate to acuminate
Shape of indeterminate branch apex	rounded	rounded, retuse to emarginate	broadly and bluntly tridentate	acute to tridentate	tridentate	tridentate
Dentate proximal axis margins	x	x	x	x	✓	✓
Branching pattern	irregularly & sparsely branched	irregularly & sparsely branched	pinnate, sparse indeterminate branches	compound pinnate	compound pinnate, axes procurrent	compound pinnate, axes procurrent

CHAPTER 4

SPONGE-ALGAL ASSOCIATIONS IN SPECIES OF *PTILOPHORA* (GELIDIACEAE, RHODOPHYTA)

INTRODUCTION

Sponge-seaweed associations have been reported in detail for a number of macroalgal genera (Phillips 2002, Price *et al.* 1984, Rützler 1990, Scott *et al.* 1984, Zea & Weerdt 1999), in the genus *Ptilophora* Kützing they have merely been noted (Norris 1991 & 1987a). Papenfuss (1940) confirmed that the hyaline bristles covering the thalli of three *Ptilophora* species, as noted by previous authors (Kützing 1847, Harvey 1855, Agardh 1876, Engler & Prantl 1897), were in fact spicules of an encrusting sponge. To date this association has been reported in *Ptilophora diversifolia* (Suhr) Papenfuss, *Ptilophora pinnatifida* J. Agardh, *Ptilophora prolifera* (Harvey) J. Agardh and *Ptilophora spissa* (Suhr) Kützing (Norris 1987a, 1992; Papenfuss 1940), although only the degree of the sponge-coverings on algal thalli have been described. Norris (1987a) maintained that there was a relationship between the presence of surface proliferations and epiphytic sponges in *Ptilophora,* having observed their co-occurrence in three species of the genus. He speculated that the formation of surface proliferations was likely to be stimulated by the presence of the associated sponges. He further expounded this idea (Norris, 1991) in a discussion of surface proliferations in *Osmundaria prolifera* Lamouroux (Rhodomelaceae), although it was pointed out by Phillips (2002) that this hypothesis has never been tested experimentally, nor has a mechanism been suggested whereby the alga might be induced by the animal to produce proliferations. From the various reports on sponge-seaweed associations, the following categories of relationships can be distinguished:

Non-structural relationships involving endozoic algae and sponge hosts

Here the algal thallus is endozoic in a sponge that has its own distinctive architecture and basal holdfast, it being unlikely that the alga plays any structural role in the association. Examples are *Audouinella spongicola* (Weber-van Bosse) Stegenga (Acrochaetiaceae) from Western Australia (Woelkerling and Womersley, 1994) and *Ostreobium* cf. *constrictum* Lucas (Ostreobiaceae) from shallow patch reefs in Belize (Rützler, 1990). Both are microscopic and filamentous algae that grow in *Mycale laxissima* Duchassaing

& Michelotti (Mycalidae) in Belize (Rützler, 1990). The algal filaments are densely intertwined and fully embedded in strands of spongin fibers, their growth resulting in the physical separation, but not chemical dissolution, of the spongin. The interface of the association shows no sign of interpenetration. As the algae retain their chloroplast pigmentation, it is probable that they remain autotrophic. *Audouinella spongicola* has been found in various sponges from the subtidal on the east and west coasts of South Africa (Stegenga *et al.*, 1997), hence, the interaction does not seem to be species-specific for the alga at least in this case.

Structural relationships involving epiphytic sponges and algal hosts

The sponge determines the overall shape of the association

The algal thallus is entirely enveloped by a sponge that determines the overall shape of the association. Two relationships of this type involve geniculate coralline algae from the subtropical western Atlantic. *Dysidea janiae* Duchassaing and Michelotti (Dysideidae) augments its skeletal fibres by incorporating *Jania adhaerens* Lamouroux (Corallinaceae), and *Xytopsues osburnensis* George and Wilson (Phoriospongiidae) reinforces its skeleton with fronds of *Jania capillacea* Harvey (Corallinaceae) (Rützler, 1990). The thalli of these algae are tough and rigid, yet pliable due to their articulations. *Dysidea janiae* has not been found without *J. adhaerens* and the association between *X. osburnensis* and *J. capillacea* is facultative.

The alga determines the overall shape of the association

Here the sponge covers the algal thallus to varying degrees, from small patches to the entirely thallus, in a thin encrustation to totally enveloping only the major branches or the whole upright. In some cases the alga may produce certain structures that provide small compartments where sponges can become established and later grow to envelop larger portions of the algal thallus.

An example is the symbiosis between *Ceratodictyon spongiosum* Zanardini (Lomentariaceae) and *Haliclona cymiformis* Esper (Chalinidae) as recorded from shallow tropical waters of the Indo-Pacific region (Grant & Hinde, 1999). The alga has a stiff, branched thallus which provides the underlying rigid skeletal structure of the association and determines its shape (Vacelet, 1981). Anastomosing branches result in a reticulum, with much of the sponge lying in the interstices (Price *et al.*, 1984) as well as forming a thin layer around the outside of the aggregated algal axes (Trautman, 1999). Only the reproductive parts and very tips of axes are exposed (Price and Kraft, 1991). In unialgal culture, however, *Ceratodictyon spongiosum* does not produce anastomosing branches (Price *et al.*, 1984). According to Norris (1987b), the reticulate thallus probably develops secondarily and may be caused by the sponge symbiont. The alga has not been found growing alone under natural condition (Trautman *et al.*, 2000) where the reticulate structure is consistently produced (Price and Kraft, 1991); thus, what causes the thallus to reticulate is still uncertain. Grand & Hinde (1999) and Davy & Hinde (1999) found that there is an exchange of algal photosynthates and sponge metabolites. Trautman (1999) and Grant & Hinde (1999), however, propose that the interaction is not primarily nutritional, but rather structural. As neither symbiont has been found growing separately in nature, it appears that the symbiosis is an obligate one (Trautman *et al.*, 2000).

Other examples are found in the frondose genera of *Thamnoclonium* Kützing (Halymeniaceae), *Codiophyllum* Gray (Halymeniaceae) (Scott *et al.*, 1984), and the species *Epiglossum smithae* (J.D. Hooker *et* Harvey) Kützing (Rhodomelaceae) and *Osmundaria prolifera* (Norris 1991, Phillips 2002). In all such cases it appears that the sponge is epiphytic rather than parasitic, since neither organism penetrates the surface of the other and the host-epiphyte relationships are highly species-specific (Phillips, 2002). In *Thamnoclonium dichotomum* (J. Agardh) J. Agardh the frond axes are covered by irregularly contoured excrescences that are covered by *Terpios symbioticus* Hentschel (Suberitidae), the spicules of which are orientated perpendicularly to the thallus surface. Reproductive leaflets, however, remain sponge free. Species of *Codiophyllum* Gray produce networks of anastomosing filiform laterals which create compartments analogous to those of *Ceratodictyon*, which a currently unidentified sponge fills. There is no evidence that the sponge in any way determines the form of the alga (Scott *et al.*, 1984). *Epiglossum smithae* always has a sponge epiphyte (Phillips, 2002) and *Osmundaria prolifera* is often associated with a sponge that can vary in thickness (Norris, 1991). Both algal species have dense, morphologically distinct proliferations from their frond surfaces. *Osmundaria prolifera* specimens which do not have a sponge epiphyte covering the surface either wholly or in part do not produce surface proliferations (Norris, 1991), whereas non-proliferate basal branches in *E. smithae* may carry sponge (Phillips, 2002). It is thought that surface proliferation may aid the sponge in bonding to the alga since the sponge can become engrained between the proliferations improving its resistance to removal by physical disturbances such as water movement.

There are cases where algae do not produce any obvious structures that may aid in sponge bonding, but have sponge epiphytes nonetheless, such as *Carpopeltis spongeaplexus* from Western Australia (Womersley and Lewis, 1994). This alga is characterized by flattened, subdichotomous axes that are almost entirely covered by the sponge *Oplitaspongia* sp., with just the ends of terminal branches extending beyond the enveloping sponge (Scott *et al.* 1984; as *Codiophyllum dicipiens*). In the Colombian Caribbean, *Haliclona* (*Haliclona*) *epiphytica* Zea & Weerdt (Chalinidae) grows on algae in the rocky intertidal to shallow-subtidal or near sandy beaches in sites of turbid water and relatively strong water movement. The hosts have been identified as *Laurencia poiteaui* (Lamouroux) Howe (Rhodomelaceae), *Bryothamnion seaforthii* (Turner) Kützing (Rhodomelaceae) and *Cryptonemia crenulata* (J. Agardh) J. Agardh (Halymeniaceae) (Zea & Weerdt, 1999), algae with cylindrically branched or flattened blades.

Definition of terms and aims

The term 'surface proliferations', or merely 'proliferations', has been used previously by Norris (1987, 1991, 1992) and Phillips (2002) to refer to any short branch issuing from the surface of a flattened or compressed blade in a distichously branched thallus such as occur on the midribs or alae in *Ptilophora*. In this study the term is used in the same way.

The surface proliferations of seven species of *Ptilophora* were described in Chapter 2, some illustrated, although the range in surface proliferation morphology was not documented.

The aims of this chapter are:

1) to improve our understanding of the sponge-algal association in *Ptilophora* by determining how widespread the phenomenon is and categorising the type of association in comparison to other reported cases of sponge-algal associations;
2) to illustrate the range of surface-proliferation morphology in the genus; and
3) to critically evaluate the hypothesis that there is a direct causal relationship between the presence of surface proliferations and the presence of sponge in species of *Ptilophora*.

MATERIALS AND METHODS

Eighty eight specimens of sixteen *Ptilophora* species were examined (Table 4.1), the one omission being the Japanese *Ptilophora irregularis* (Akatsuka & Masaki) R.E. Norris. The extent to which surface proliferations and encrusting sponges occurred on the thalli was determined. Specimens were collected along the KwaZulu Natal Province coastline (South Africa) during four expeditions that took place between 1999 and 2002. Specimens were also borrowed from local and international herbaria. Eight type specimens were examined as well as specimens from several type localities. The majority of specimens studied were dried (pressed), though a few were preserved in 5% formalin in seawater or dried in silica gel. The number of specimens observed for each species varied due to their availability (with some species only being known from type collections).

Specimens were categorised into four classes depending on the presence or absence of surface proliferations and sponge epiphytes. A χ^2 goodness of fit was performed on the data to test the null hypothesis that sponge epiphytes are as likely to occur on proliferate thalli as on non-proliferate thalli. Observations were made using a Wild stereo dissecting photomicroscope and photos were taken with an Olympus Camedia digital camera.

Table 4.1. Details of specimens of fifteen species of *Ptilophora* observed in this study.

Species	Total number of specimens observed	Origin of specimens observed	Specimen collection locations
P. prolifera	13	F.C., MELU	Australia
P. diversifolia	9	BOL, F.C., GENT	South Africa
P. spissa[1]	1	W	South Africa
P. biserrata[1]	4	C	Mauritius
P. pterocladiodes[1]	2	P	Madagascar
P. mediterranea[1]	1	P	Greece
P. pinnatifida[1]	11	BOL, F.C., GENT, LD	South Africa
P. scalaramosa	2	MELU, F.C.	Philippines
P. subcostata	6	LD, S,	Japan
P. coppejansii	4	F.C., GENT	South Africa
P. rhodoptera	3	F.C., GENT	South Africa
P. leliaertii	10	F.C., GENT, GRA	South Africa
P. helenae	6	GRA, BOL	South Africa
P. hildebrandtii[1]	11	F.C., GENT, L	South Africa, Kenya
P. rumpii[1]	2	BM, NU	South Africa
P. pectinata[1]	4	BM, F.C., GRA	South Africa, Australia

[1] Type specimens were observed. F.C. – personal field collections including specimens collected worldwide by D.W. Freshwater. Herbarium codes: BM – The Natural History Museum, England; BOL – University of Cape Town, South Africa; C – University of Copenhagen, Denmark; GENT – University of Gent, Belgium; GRA – Albany Museum, South Africa; L – Nationaal Herbarium Nederland, Leiden University branch, Netherlands; LD – Botanical Museum (Lund), Sweden; MELU – University of Melbourne, Australia; NU – University of Natal, South Africa; P – Muséum National d'Histoire Naturelle (Paris), France; S – Swedish Museum of Natural History (Stockholm), Sweden; W - Naturhistorisches Museum Wien, Austria.

Transverse sections were cut from pressed specimens of *P. diversifolia*, *P. spissa* and *P. coppejansii* Tronchin and preserved in a 50% Karo™ solution. From these the physical integrity of the interface between sponge epiphyte and algal cortex was examined. Photos were taken with an Olympus DP50 digital camera mounted on a Leitz Diaplan compound microscope.

Sponge epiphytes were identified from four species of *Ptilophora*. Fragments of sponge containing choanosomes and ectosomes were cut from voucher specimens and placed in Eppendorf tubes. The spicules were isolated from the sections in a fume cupboard by digesting the sponge tissue in 100% nitric acid. Material was then rinsed three times (twice with distilled water and once with 70% ethanol) prior to microscopic examination.

Between each rinse the material was centrifuged for 3-5 min at 3000 rpm. Clean spicule samples were stored in 100% ethanol at room temperature, resuspended and pipetted onto a microscopic slide, the ethanol allowed to evaporate off on a heated tray at 40°C. Once completely dry, they were stained with Canada Balsam. To examine the skeletal arrangement of the sponge, perpendicular sections of sponge-encrusted algal branches were cut from the voucher material, processed automatically through a series of dehydrating and embedding agents, and embedded in paraffin wax. Histological sections of approximately 75 µm were cut using a microtome, the wax removed by washing in histosol (Hooper, 1996).

RESULTS

Due to the non-standardized number of specimens examined for each species, the numbers scored in Table 4.2 merely indicate the occurrence of surface proliferations and sponge in those species rather than statistical significances.

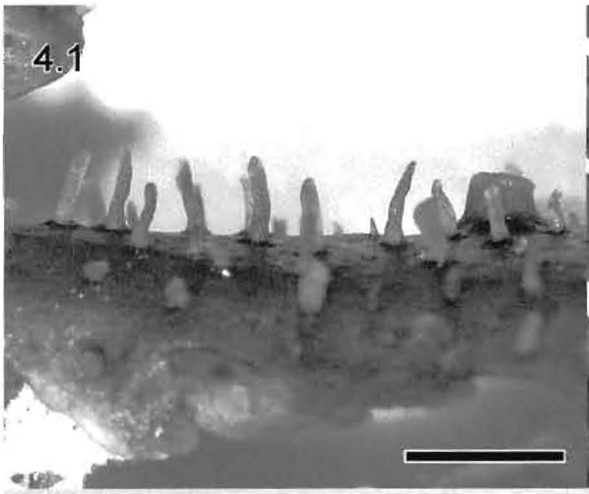
Fourteen of the sixteen species observed produced surface proliferations with a variety of morphologies (Table 4.2) ranging from simple cylinders (Fig. 4.1) to ligulate, subcylindrical or flattened proliferations (Fig. 4.2), often with tripartite apices and occasionally becoming pinnately branched to varying degrees (Fig. 4.3). *Ptilophora diversifolia* and *P. spissa* were similar in producing scale-like proliferations. In *P. diversifolia* these were sometimes eroded, leaving a series of parallel ridges on the alae adjacent to the blade margins (Fig. 4.4). In a few species, surface proliferations were rhizoidal in nature, either minute spurs (Fig. 4.5), probably representing rhizoid primordia or long, relatively unbranched and lightly pigmented outgrowths (Fig. 4.6). Surface proliferations are present but have not been previously reported for *Ptilophora biserrata* (Børgesen) R.E. Norris, *Ptilophora helenae* (A. & E.S. Gepp) R.E. Norris *Ptilophora pterocladioides* Andriamampandry, *Ptilophora rumpii* (Dickinson) R.E. Norris, and *Ptilophora subcostata* (Okamura) R.E. Norris. The surface proliferations in *P. subcostata* occurred on the proximal part of the axis and, except for one adventitious determinate branch, were all rhizoidal in nature. *Ptilophora biserrata* and

Table 4.2. The extent of surface proliferations (SP) and sponge epiphytes on the surface of fronds of *Ptilophora* species, and the type of SP produced by each species.

Species	Produces SP	Figure no. illustrating type of SP produced	Number of specimens with both SP and sponge[2]	Number of specimens with sponge and no SP	Number of specimens with SP and no sponge	Number of specimens with neither SP nor sponge	Specimens always with minor sponge association only (<10% cover)
P. prolifera	✓	1-3	10 (9)		1	2	
P. diversifolia	✓	1, 2, 4	7 (3)		2		
P. spissa	✓	1, 2, 4	1 (1)				
P. biserrata	✓	2	3 (3)	1			✓
P. pterocladiodes	✓	2	1			1	✓
P. mediterranea		n/a				1	
P. pinnatifida	✓	1-3	3 (3)	1	2	5	
P. scalaramosa	✓	5, 6			2		
P. subcostata	✓	3, 6	1	1	1	3	✓
P. coppejansii[1]	✓	1-3	4 (1)				
P. rhodoptera	✓	1,2	3 (1)				
P. leliaertii[1]	✓	1-3, 6	4 (3)	6			
P. helenae	✓	2, 3	1		5		✓
P. hildebrandtii	✓	1-3	5 (4)	1	5		
P. rumpii	✓	2,3	1 (1)	1			✓
P. pectinata		n/a				3	✓
Totals for species	**14**		**13**	**6**	**7**	**6**	**5**

[1] *P. coppejansii* and *P. leliaertii* were referred to as *Ptilophora* sp. and *Beckerella* sp. respectively in chapter 2

[2] Numbers in brackets indicate the number of these specimens that produced surface proliferations in regions of the thallus that were not covered by sponge.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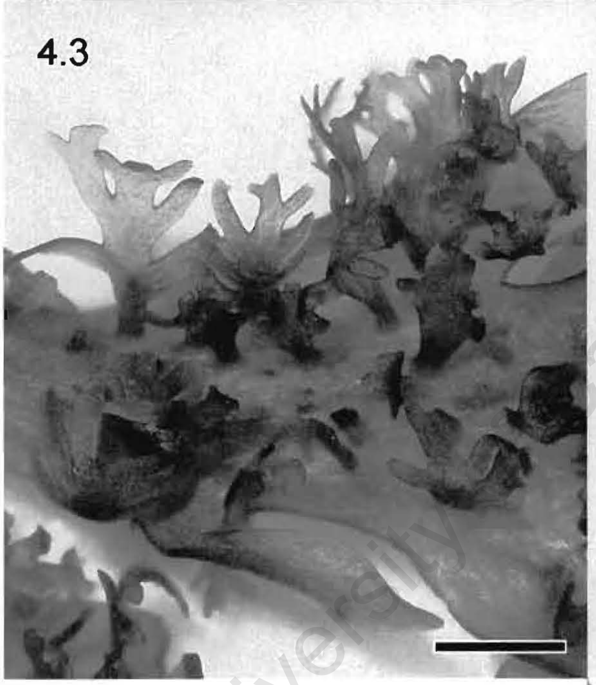
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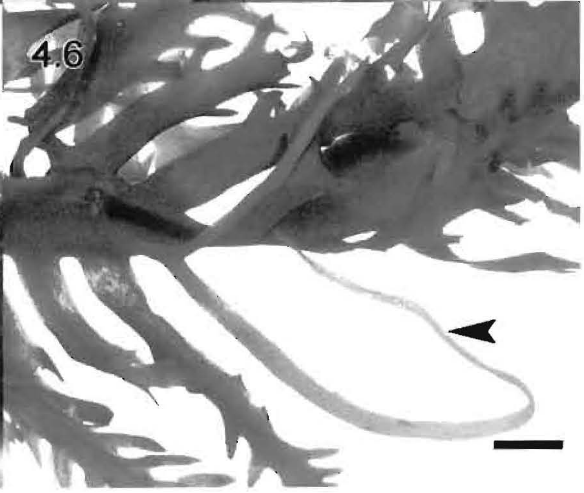
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Fig. 4.1. Marginal view of flattened branch of *Ptilophora prolifera* with short cylindrical proliferation issuing from the convex surface. Scale = 1 mm. Fig. 4.2. Ligulate proliferations issuing from the midrib of *Ptilophora hildebrandtii*. Scale = 1 mm. Fig. 4.3. Pinnately branched proliferations borne on the surface of a distal branch of *Ptilophora prolifera*. Scale = 1 mm. Fig. 4.4. Scale-like, flattened surface proliferations issuing from the midrib of *Ptilophora diversifolia*. These develop on the ala as well but are often eroded, leaving a marginal series of parallel ridges as shown here. Scale = 1 mm. Fig. 4.5. Marginal view of the lower axis of *Ptilophora scalaramosa* from which minute spurs are borne which are probably the primordia of adventitious rhizoids. Scale = 1 mm. Fig. 4.6. A branched, adventitious rhizoid issuing from the surface of a major branch of *Ptilophora scalaramosa*. Scale = 1 mm.

P. pterocladioides produced surface proliferations very infrequently (about three surface proliferations per plant) and only a few surface proliferations were found scattered on major branches in *P. rumpii*. *Ptilophora helenae* (Dickinson) Norris had a few surface proliferations issuing from the midrib (mostly on the proximal axis) and from sites of injury (circular recesses) on the midrib.

Thirteen species produced both surface proliferations and sponge associations, some consistently but others occasionally (Table 4.1). The χ^2 test performed on the data found significantly more proliferate thalli with a sponge epiphytes ($P < 0.001$). Sponge epiphytes were generally bonded to the surface proliferations and the blade surface in the interstices between surface proliferations (Fig. 4.7). However, surface proliferations sometimes developed in parts of the algal thallus without a sponge, whereas some specimens also had sponge epiphyte bonded to non-proliferating parts of the thallus. *Ptilophora rhodoptera* R.E. Norris was particularly notable in this respect since it bore very few surface proliferations but was almost entirely covered in a thin layer of sponge. Sponges bonded comparatively weakly in non-proliferating parts of its thalli, especially on cylindrical axes. Some specimens with sponge epiphytes had no surface proliferations, and vice versa, and a few specimens were encountered that lacked both surface proliferations and epiphytic sponge entirely. All sponge-encrusted specimens had normal pigmentation regardless of the thickness of the sponge cover and surface or lateral proliferations bearing fertile structures were noted to always extend beyond the algal cover.

Six species had only a minor association with sponge with never more than 10% of their thalli covered (Table 4.1). All six are similar in producing surface proliferations relatively infrequently on any single thallus and having narrow blades that are either sparsely branched, compound pinnate, or tending towards these morphologies by virtue of receded alae and simple, linear pinnae as in *P. rumpii.* Five other compound pinnate species are *P. helenae, P. hildebrandtii* (Hauck) R.E. Norris, *P. pectinata*, *P. scalaramosa* (Kraft) R.E. Norris and *P. leliaertii* Tronchin. *Ptilophora hildebrandtii* had the more conspicuous sponge association, although it was concentrated proximally on the axis and primary branches where surface proliferations tend to be localized. Only one specimen of *P. helenae* carried a sand-binding sponge epiphyte in a small patch. *Ptilophora pectinata* and *P. scalaramosa* were never found with a sponge epiphyte. *Ptilophora leliaertii* has compound pinnate fronds with fairly narrow, concave branches. Sponge epiphytes are often situated in the concavities and can spread to engulf the branch.

Two specimens of *P. coppejansii* were completely enveloped in a sponge except for some distal branch ends, which served in the identification of the specimens (Fig. 4.9). The sponge was carefully removed from a basal portion of the frond to reveal the underlying algal thallus (Fig. 4.11) which was aberrant in that there were numerous proliferations with closely spaced lateral branches and an irregular branching pattern in lower order branches. The thallus was only partially distichous as branches in places developed in two, rather than one plane. Lateral branches were covered in either tufts of digitate or pinnate surface proliferations, and reticulating lateral proliferations were also produced (Fig. 4.10). Some lateral branches were also finely branched with subcylindrical rather than flattened branches, while others had abnormally broad, contoured and abruptly terminated apices (Fig. 4.11). A formalin-preserved specimen was bleached and viewed against strong backlighting in order to see the underlying algal thallus through its sponge encrustation. The ends of distal branches were observed to have the normal dentate morphology, pinnule shape and branching pattern of this species (Fig. 4.9). A specimen of *P. leliaertii* was found with an aberrant morphology similar to that observed in *P. coppejansii*, with fine, reticulating lateral proliferations (Fig. 4.8), but it altogether lacked a sponge epiphyte.

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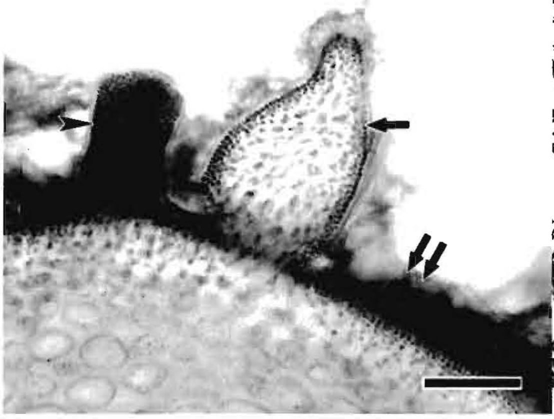

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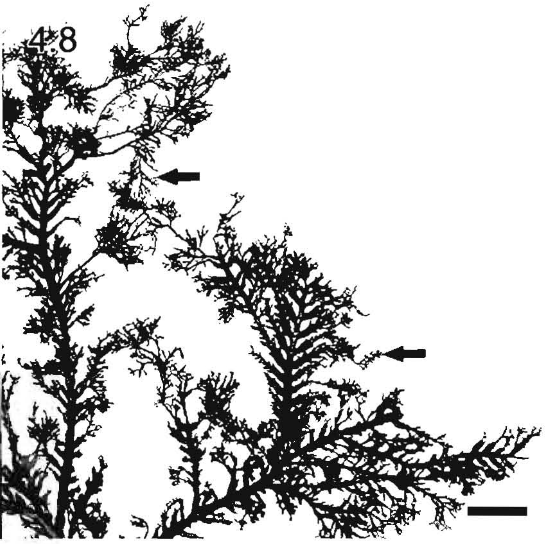

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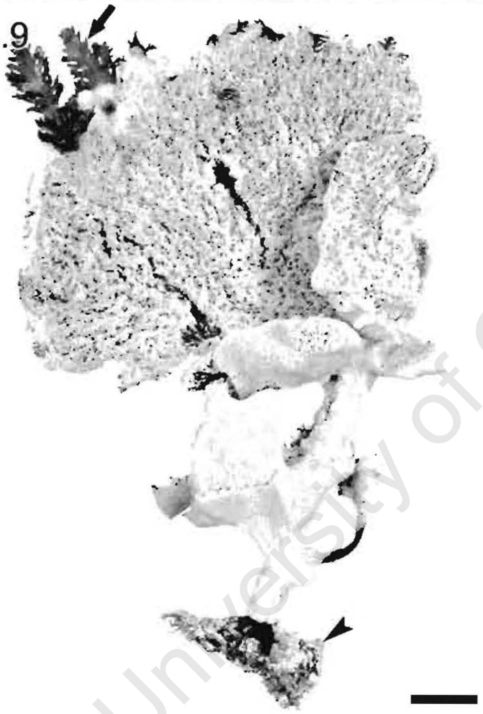

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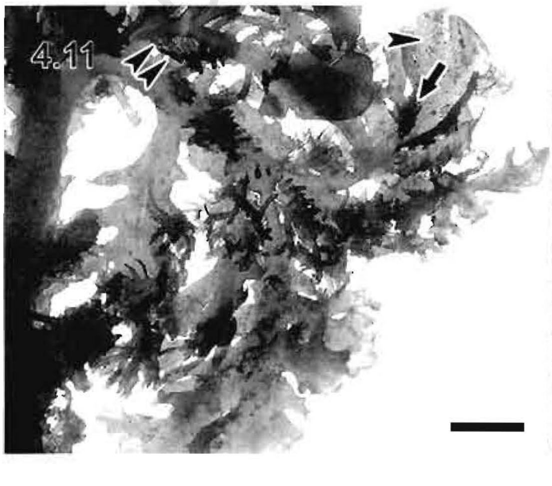

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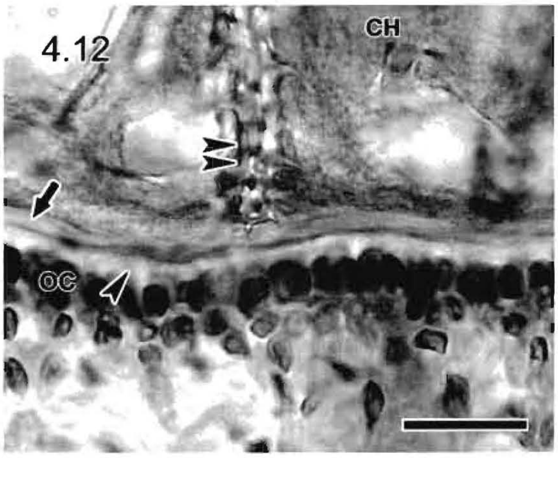


Fig. 4.7. Transverse section through the midrib of a lateral branch of *Ptilophora diversifolia* coated in a sponge epiphyte (arrows). The section cuts longitudinally through a surface proliferation (arrow) and a second proliferation lies in the background (arrowhead). Scale = 100 µm. Fig. 4.8. Distal branches of *Ptilophora leliaertii* bearing narrow, reticulating lateral proliferations (arrow). Scale = 1 cm. Fig. 4.9. Sponge-encrusted *Ptilophora coppejansii* specimen where sponge epiphyte forms a fan uniting the branches of the underlying algal thallus. Un-encrusted distal branch ends have the normal branch morphology (arrow). The holdfast (arrowhead) is encrusted in sand. Scale = 1 cm. Fig. 4.10. Reticulating lateral proliferation of a specimen of *Ptilophora coppejansii* enveloped in sponge. Most of the sponge covering was removed though some still remains between branches (arrow). Scale = 1 mm. Fig. 4.11. Specimen of *Ptilophora coppejansii* with thick sponge cover removed. Sponge spicules remain in places (arrowhead). Surface proliferations issue frequently from branches, and simple or pinnate proliferations may sometimes occur in tufts (arrow). Fine, prolific and subcylindrical branches often present (arrowheads). Scale = 3 mm. Fig. 4.12. Detail of the sponge-algal interface in transverse section of a sponge-encrusted branch of *Ptilophora diversifolia*. Acanthostyles (arrowheads) are arranged perpendicularly to the surface of the algae within the choanosome (CH) of the sponge. There is no sign of disruption to or interpenetration of the outer cortex (OC) of the alga, and the cuticle (arrowhead) is intact. A band of spongin fibres (arrow) bonds the sponge to the algal surface. Scale = 20 µm.

In transverse sections of sponge-encrusted algal branches, the interface of the association showed no sign of interpenetration (Fig. 4.12). The cuticle surrounding the algal cortex was always intact and merely covered by sponge.

Eight sponge species in seven genera were identified on four species of *Ptilophora*, with up to three sponge species epiphytic on the same specimen of *P. diversifolia* and two species on the same specimen of *P. coppejansii* (Table 4.3). *Halichondria cf. panicea* Pallas (Halichondriidae) and *Ophlitaspongia sp.* (Microcioniidae) were both found on specimens of *P. diversifolia* and *P. coppejansii.*

Table 4.3. List of sponge epiphytes on four species of *Ptilophora*.

Species of *Ptilophora*	Specimen coll. Location	Sponge epiphyte
P. diversifolia (specimen 1)	Protea Banks, South Africa	*Tedinia (Tedania) sp.* (Tedaniidae) *Halichondria cf. panicea* Pallas (Halichondriidae) *Ophlitaspongia sp.* (Microcioniidae)
P. diversifolia (specimen 2)	Protea Banks, South Africa	*Myxilla (Myxilla) simplex* Baer (Myxillidae) *Tedinia (Tedania) anhelans* Lieberkuhn (Tedaniidae)
P. pinnatifida	The Kowie, South Africa	*Isodictya cf. multiformis* Stephens (Isodictyidae)
P. prolifera	Penguin Island, Australia	*Mycale (Mycale) sp.* (Mycaliidae)
P. coppejansii (specimen 1)	Protea Banks, South Africa	*Haliclona (Gellius) sp.* (Chaliniidae)
P. coppejansii (specimen 2)	Protea Banks, South Africa	*Halichondria cf. panicea* Pallas (Halichondriidae) *Ophlitaspongia sp.* (Microcioniidae)

DISCUSSION

Surface proliferations occur on all *Ptilophora* species except *P. irregularis*, *P. mediterranea* (H. Huvé) R.E. Norris and *P. pectinata*. However, it is possible that these three can also produce these structures since only limited material of *P. mediterranea* and *P. pectinata* was available for study. In fact, Akatsuka and Masaki (1983) state that *P. irregularis* occasionally produces adventitious branches or axes from the surface of the frond.

Sponge epiphytes are facultative in at least some species and the occurrence of surface proliferations on branches without a sponge epiphyte, and vice versa, strongly suggests that there is no direct causal relationship between surface proliferations and the presence of epiphytic sponges. Phillips (2002) has commented on the unlikelihood that proliferations in *Epiglossum smithiae* are induced by the sponge epiphyte as basal non-proliferate blades are usually present. An ultrastructural investigation of the interface of similar host-epiphyte associations in *Thamnoclonium* and *Codiophyllum*, Scott *et al.* (1984) also found the interface to be structurally unmodified.

There does appear, however, to be a relationship between the presence of surface proliferations and associated sponges as noted by Norris (1987) for a significant number of proliferate thalli in several diverse red algal groups consistently epiphitised by sponges. It is likely that sponges exploit a niche habitat provided by the algae, surface proliferations enabling them to bond well and resist removal by water movement. This has also been suggested as the reason for sponge growth on *Codiophyllum* and *Thamnoclonium* by Scott *et al.* (1984) and could also be true for the associations in *Ceratodictyon spongiosum* (Price and Kraft, 1991), *Epiglossum smithii* and *Osmundaria prolifera* (Phillips, 2002). It appears that sponges bond relatively weakly to cylindrical non-proliferate axes in plants of some species such as *Thamnoclonium dichotomum* Scott *et al.* (1984), which lends support to this hypothesis.

The sponge association in *Ptilophora* is also similar to the associations documented in *Carpopeltis spongeaplexus*, *Codiophyllum*, *Thamnoclonium* (Womersley and Lewis, 1994) and *Ceratodictyon spongiosum* (Price and Kraft, 1991) in that fertile structures are borne on proliferations or terminal branch tips that extend beyond the sponge cover. The occurrence of sponges on *Ptilophora* species may thus be determined by a number of factors, including overlapping distributions and habitats of the pairing organisms. Considering the scarcity of surface proliferations and extensive sponge epiphyte cover in the broad-bladed *P. rhodoptera*, as well as the minor sponge associations in several scarcely branched and pinnate species with narrow blades, there may also be a relationship between the presence and degree of cover of sponge epiphytes and the breadths of the host blades. Fronds with narrow or receded alae may not provide sufficient substratum for sponge establishment, possibly because of the reduced surface area. *P. leliaertii* is particularly interesting in this respect since, despite the fact that it has fairly narrow, compound pinnate fronds; the concave profiles of the branches appear to provide a protective habitat for sponge epiphytes. Such morphology apparently also favours sponge attachment to the smooth frond of the Western Australian *Curdiea irvineae* J. Agardh of the Gracilariales (Womersley, 1966).

In the *Haliclona (Haliclona) epiphytica* / *Laurencia poiteaui* association which is abundant in wave-surge areas (Little and Littler, 2000), the alga lacks structural modifications likely to aid in sponge bonding, suggesting that more than just its branch morphology is responsible for the pairing, although it is unknown what influence the habitat has on the morphology. In the case of *Ptilophora*, the erect, distichous thalli in turbid and rapidly moving water would seem to provide an optimal substratum for a filter-feeding organism to occupy. Five sponge-associated species of *Ptilophora*, as well as *Thamnoclonium dichotomum* and *Codiophyllum natalense* (Gray), occur together at Protea Banks, a deep offshore reef (18-37m) on the eastern coast of South Africa. These beds are situated on open reef flats periodically exposed to a strong prevailing current in which the distichous algal thalli extend upwards into the water column. The water is often turbid and carries organic particles from the numerous estuaries along this part of the coastline. It is perhaps not surprising then that the species often encountered

Fig. 5.1. Worldwide distribution of *Ptilophora.*

new species *Ptilophora pinnatifida* J. Agardh which lacked surface proliferations entirely. Schmitz (1894) thought that the presence of proliferations was a poor generic character, a notion also supported much later by Dickinson (1950), and returned *Ptilophora prolifera* (Harvey) J. Agardh and *P. pinnatifida* to *Gelidium* Lamouroux. Subsequently, phycologists began to place more emphasis on the vegetative structure of *Ptilophora* as a diagnostic character. Holmes (1896) described a new species *Ptilophora beckeri* Holmes which lacked surface proliferations, placing it in this genus because of its four-layered vegetative structure and the characteristic infra-cortical layer of rhizine filaments. He also suggested recognising a separate section of *Ptilophora* to incorporate *Gelidium subcostatum* Okamura ex Schmitz, *Ptilophora prolifera* (Harvey) Schmitz and *P. beckeri*, species characterised by flattened fronds that lacked surface proliferations. Engler & Prantl (1897) redescribed *Ptilophora* and continued to use the presence of scale-like proliferations and stiff, hard spines as major diagnostic characters for *Ptilophora*, based on the type species *P. spissa*. Similarly, Papenfuss (1940) attached major taxonomic importance to the presence of surface proliferations in *Ptilophora* but found that the bristles on the thalli of *P. spissa* and *P. diversifolia* (Suhr) Papenfuss were sponge spicules. Papenfuss also re-incorporated *Gelidium proliferum* into *Ptilophora* for its striking resemblance to *P. diversifolia* and *P. spissa*. Dickinson (1950) provides perhaps the best example of the uncertainty regarding the diagnostic characters of *Ptilophora*, as she debated whether to place two new species (*Gelidium helenae* Dickinson and *Gelidium rumpii* Dickinson), that lacked surface proliferations but had a four-layered vegetative structure, into *Ptilophora* or *Gelidium*. He placed these species into *Gelidium*, having found nothing in the cystocarp morphology to distinguish them from *Gelidium*, and having noticed that *Gelidium* contained some midribbed plants [*G. subcostatum* and *G. pinnatifidum* (J. Agardh) Schmitz] citing De Toni (1897). The species were only incorporated into *Ptilophora* much later (Norris, 1987a).

Kylin (1956) delimited a new genus *Beckerella* based on *Ptilophora pinnatifida*, characterised by a lack of surface proliferations and the presence of a midrib in basal parts that disappeared distally. Into this genus he also added *P. beckeri*, *Gelidium subcostatum* and *Thysanocladia hildebrandtii* Hauck. He stated that *Ptilophora* was characterised by a more distinct midrib and the presence of scale-like proliferations on the midrib and frond surface. Fan (1961) transferred four more species into *Beckerella*: *Gelidium biserratum* Børgesen, *Gelidium helenae* Dickinson, *Pterocladia pectinata* (A. & E.S. Gepp) Fan *et* Papenfuss and *Gelidium rumpii* Dickinson. He found all the species in this group to have the same system of apical growth and branch development. The subsequent addition of three new species to *Beckerella*, namely *B. meditteranea* Huvé [including *Phyllophora aegei* Giaccone sp. nov. in Giaccone (1968) as taxonomic synonym (Athanasiadis, 1987)], *B. irregularis* Akatsuka *et* Masaki and *B. scalaramosa* Kraft, made the diagnostic characters of the genus appear somewhat tenuous. These species had fundamental differences to other *Beckerella* species and their placement in the genus was based predominantly on the presence of a four-layered vegetative structure. *Beckerella mediterranea* was reported to produce terminal adventitious rhizoids on decumbent fronds that would produce secondary points of attachment from which new fronds would develop when they came in contact with the substratum (Huvé 1962), a system of vegetative propagation not seen previously in *Beckerella*. *Beckerella irregularis* was said to lack a midrib entirely (Akatsuka and Masaki, 1983). *Beckerella scalaramosa* was reported to produce small unbranched proliferations on the broad surfaces of lower axes, somewhat as in the genus *Ptilophora* (Kraft, 1976).

Akatsuka (1987) discussed the concept of *Beckerella*, stating that the presence of a large-celled inner cortex together with the absence of surface proliferations was diagnostic for the genus. Norris (1987a) found surface proliferations in the type species *Beckerella pinnatifida* (J. Agardh) Kylin and merged all *Beckerella* species into *Ptilophora*, except *Beckerella hildebrandtii* (Hauck) Norris which he incorporated later (Norris 1990b). Norris (1987a) regarded the four-layered vegetative structure of *Ptilophora* to be the major diagnostic character, rather than proliferations, which he regarded as probably a facultative response to epiphytic sponges. The reclassification of *Beckerella* by Norris (1987a) was not widely accepted (Murase *et al.* 1989; Silva *et al.* 1996; Trono 1997; Barreto *et al.* 1997; Kraft *et al.* 1999). Akatsuka (1987) and Athanasiadis (1987) recognised *Beckerella*, but were probably unaware of Norris's (1987a) proposed change as all three papers were published in the same year. Silva *et al.* (1996) provisionally accepted both genera pending further input from other phycologists. He made a new combination, *Beckerella pterocladioides* (Andriamampandry) P. Silva, for a species originally proposed under this name in a thesis (Andriamampandry, 1976) and later validly published as *Ptilophora pterocladioides* Andriamampandry (Andriamampandry, 1988) as the result of a last-minute change of name in keeping with Norris's merger of the two genera (Silva *et. al.*, 1996).

The aim of this chapter is to provide a review of the diversity and distribution of *Ptilophora* including a key to the species and a photographic plate, brief diagnosis, and note on the distribution of each species. A review is warranted for the following reasons: a) apart from the brief review of *Beckerella* by Akatsuka (1987) which included a key to the species, numerous illustrations and a discussion on a segregating character, a comprehensive review of *Ptilophora* has not been done before; b) when Norris (1992b) merged *Ptilophora* with *Beckerella*, a key to all the species was provided but only those occurring in South Africa were reviewed; c) a number of changes have since been made to the genus (Andriamampandry 1988, Norris 1990b, Silva 1996, chapter 3 of this thesis); and d) detail of approximately half the species of *Ptilophora* is only present in old original descriptions, some of which are difficult to access. *Ptilophora spissa* is placed in synonymy with *P. diversifolia* for which a neotype specimen is also designated. The cystocarpic phase of *P. rumpii* and the male phase of *P. pterocladioides* are reported for the first time. Where possible, efforts were made to illustrate alternative specimens to those illustrated in previous publications in order to provide a better understanding of intraspecific morphological variability by comparison with previous illustrations.

MATERIALS AND METHODS

An extensive literature survey was conducted. Specimens were collected using SCUBA along the east coast of South Africa during four expeditions that took place between 1999 and 2002. Specimens were also loaned from local and international herbaria (BM, BOL, GENT, GRA, L, LD, MELU, NU, P, S and W). Observations were made of all species of this genus except *Ptilophora irregularis* (Akatsuka & Masaki) Norris, for which specimens were unavailable. Type specimens of all other species as well as specimens from type localities were examined. The majority of specimens observed were dried (pressed) and a few preserved in 5% Formalin in seawater or dried in silica gel. Observations were made using a Wild stereo dissecting photomicroscope and photographs were taken with an Olympus Camedia digital camera. Transverse sections of specimens were cut by hand, stained with aniline blue stain and preserved in a 50% Karo^{TM} solution. These were photographed with an Olympus DP50 digital camera mounted on a Leitz Diaplan compound microscope. Morphometric data was gathered using image analysis software (UTHSCSA Image Tool v. 3.00). Sequence divergences between *rbc*L and LSU gene sequences generated from various *Ptilophora* species in chapter 3 were calculated using PAUP (v. 4.0b8, Swofford 2001).

TAXONOMY
RESULTS

Ptilophora Kützing in Botanische Zeitung 5: 25 (1847). Type species: *Ptilophora spissa* (Suhr) Kützing (*Phyllophora spissa* Suhr) [= *Ptilophora diversifolia* (Suhr) Papenfuss].

The currently accepted concept of *Ptilophora* is clearly defined by Norris (1987a). In summary, plants of this genus have large distichously branched fronds, generally 20-30 (-60) cm tall and up to 1 cm broad, attached by a fibrous holdfast which is often robust. Fronds usually have a distinct midrib in proximal parts, may produce surface proliferations and are often covered in places by a sponge epiphyte. The vegetative structure is diagnostic for this genus, comprising four distinct layers in transverse section including a distinct layer of densely packed rhizines between a pigmented outer cortex and well-defined, large-celled inner cortex of round to elliptical and periclinally-arranged cells, surrounding a central filamentous medulla (Fig. 5.4). Reproductive tissue is borne in marginal proliferations, rarely in surface proliferations. Although female reproductive material has not yet been reported for six of the species, the genus appears to have a '*Gelidium*-type' (bilocular) female reproductive and cystocarp system.

Sixteen species of *Ptilophora* are recognised here. The species generally lack single discrete defining characters (e.g. presence/absence of a vegetative structure) and are instead defined by a combination of external morphological characters. No reliable differences in cell dimensions and other details of the vegetative structure were found, and consequently such characters were not used in delimiting species. Fertile structures are not useful in differentiating between species, partly due to an incomplete knowledge of these structures for many of the species. Characters such as the distinctiveness of the midrib, branching pattern, pinnule shape, between-branch distances, frond width, and maximum branch order, are used to distinguish between species in the following key:

Artificial key to the species of *Ptilophora*

1.a. Branches in distal parts have flexuous alae and a distinct midrib clearly visible with the naked eye……………………………………………………………2

2.a. Fronds pinnate…………………………………………………………………3

3.a. Mature ultimate pinnae slightly cuneate, bent procurrently, with truncate to tridentate apex………………10. *Ptilophora pinnatifida*

3.b. Mature ultimate pinnae rostrate, procurrently directed with acute to rounded apex…………………………………14. *Ptilophora rumpii*

3.c. Ultimate pinnae are subulate to mucronate conferring a serrated appearance to the frond margin, adventitious rhizoids develop from distal branch apices……………………8. *Ptilophora mediterranea*

3.d. Ultimate pinnae are very closely spaced (up to 1.3 mm apart), linear, ≤3 mm long and ≤1 mm wide…………..2. *Ptilophora coppejansii*

2.b. Fronds not pinnate; margins smooth, undulate, crenate, serrate or eroded…..4

4.a. Margins bearing blunt or mucronate serrations (due to apical papillae), blades up to 1 cm broad, irregularly and sparsely branched, with

frequent lateral vegetative proliferations..............................
....13. *Ptilophora rhodoptera*

4.b. Margins smooth, undulate to slightly eroded, blades up to 5 mm broad, irregularly and sparsely branched; aberrant morphs have very eroded margins, abundant surface proliferations predominantly from the midrib, and closely spaced (often abutted) pinnae.......................................3. *Ptilophora diversifolia*

1.b. Midrib is not distinct to the naked eye in flattened distal branches..................5

5.a. Fronds irregularly and sparsely branched..6

6a. Branch and axis margins mostly serrate, midrib indistinct throughout......................................1. *Ptilophora biserrata*

6.b. Frond margins smooth except for basal branch stubs on proximal axis, midrib indistinct throughout................6. *Ptilophora irregularis*

6.c. Frond margins smooth, midrib distinct only in proximal parts, branches bear numerous, irregularly disposed constrictions
....12. *Ptilophora pterocladioides*

5.b. Fronds pinnately branched..7

7.a. Flattened branches concave..8

8.a. Flattened branches sometimes concave (pinnules normally not concave), always narrower than bearing branch, frond up to 6 mm broad, usually producing abundant surface proliferation...........................11. *Ptilophora prolifera*

8.b. Flattened branches consistently concave, up to 30% broader than bearing branch, frond up to 2.5 mm broad, surface proliferations produced very seldom or issue predominantly from lower parts.........................7. *Ptilophora leliaertii*

7.b. Flattened branches not concave...9

9.a. Distance between primary branches variable, 0.4-8 mm apart (Mean=2.4, Std. Dev.= 1.35), pinnules subulate basally conferring serrate appearance to distal branches when short (<1 mm)..............................16. *Ptilophora subcostata*

9.b. Distance between primary branches relatively uniform, 0.2-3 mm apart (Mean=1, Std. Dev.=0.45)........................10

10.a. Plants pectinate with rostrate pinnae sometimes bearing elongate terminal ligules....................11

11.a. Distance measured on primary branches from the base to the point at which ramuli commence is 1.4-10.3 mm (mean=5.2, std. dev.=1.57)...........9. *Ptilophora pectinata*

11.b. Distance measured on primary branches from the base to the point at which ramuli commence is 0.7-7.7 mm (mean=2.6, std. dev.=0.97).............4. *Ptilophora helenae*

10.b. Plants compound pinnate with linear pinnae that are very uniformly spaced and lack elongate terminal ligules..12

12.a. Up to 4 orders of branches, primary branches 0.4-3 mm apart (mean=0.93, std. dev.=0.39),

only known from the Philippines, Indonesia and northern Queensland (Australia)........
......................15. *Ptilophora scalaramosa*

12.b. Up to 6 orders of branches, primary branches 0.2-1.3 mm apart (mean=0.7, std. dev.=0.24), only known from the East African coast (Kenya to South Africa).......
.....................5. *Ptilophora hildebrandtii*

1. *Ptilophora biserrata* (Børgesen) Norris in Botanica Marina 30: 256 (1987a). Type locality: Tombeau Bay, Mauritius.
Fig. 5.2-5.4

Gelidium biserratum Børgesen, 8-11 (1943).
Beckerella biserrata (Børgesen) Fan & Papenfuss in Fan, 322 (1961).

Description

Plants up to 22 cm in length. Branches may become decumbent, reattach to the substrate and give rise to new uprights. Thallus flattened, to 300 μm thick, 1-2.5 mm broad, lacking a distinct midrib. Branching up to three orders, distichous, irregular and sparse. Blade margins with closely-spaced (1-3 mm apart), procurrent serrations developing infrequently into indeterminate branches. Adventitious branches may issue from the thallus surface. Vegetative structure is four-layered. Only sterile plants known.

Distribution

Only known from the type locality, Tombeau Bay, Mauritius (Børgesen, 1943).

Specimens examined: Holotype - Tombeau Bay, Mauritius; Vaughan; 10 ii 1939; 322 (C). Tombeau Bay, Mauritius, dredged from 80 fathoms (-146 m); Vaughan; 10 ii 1939; 274 (C).

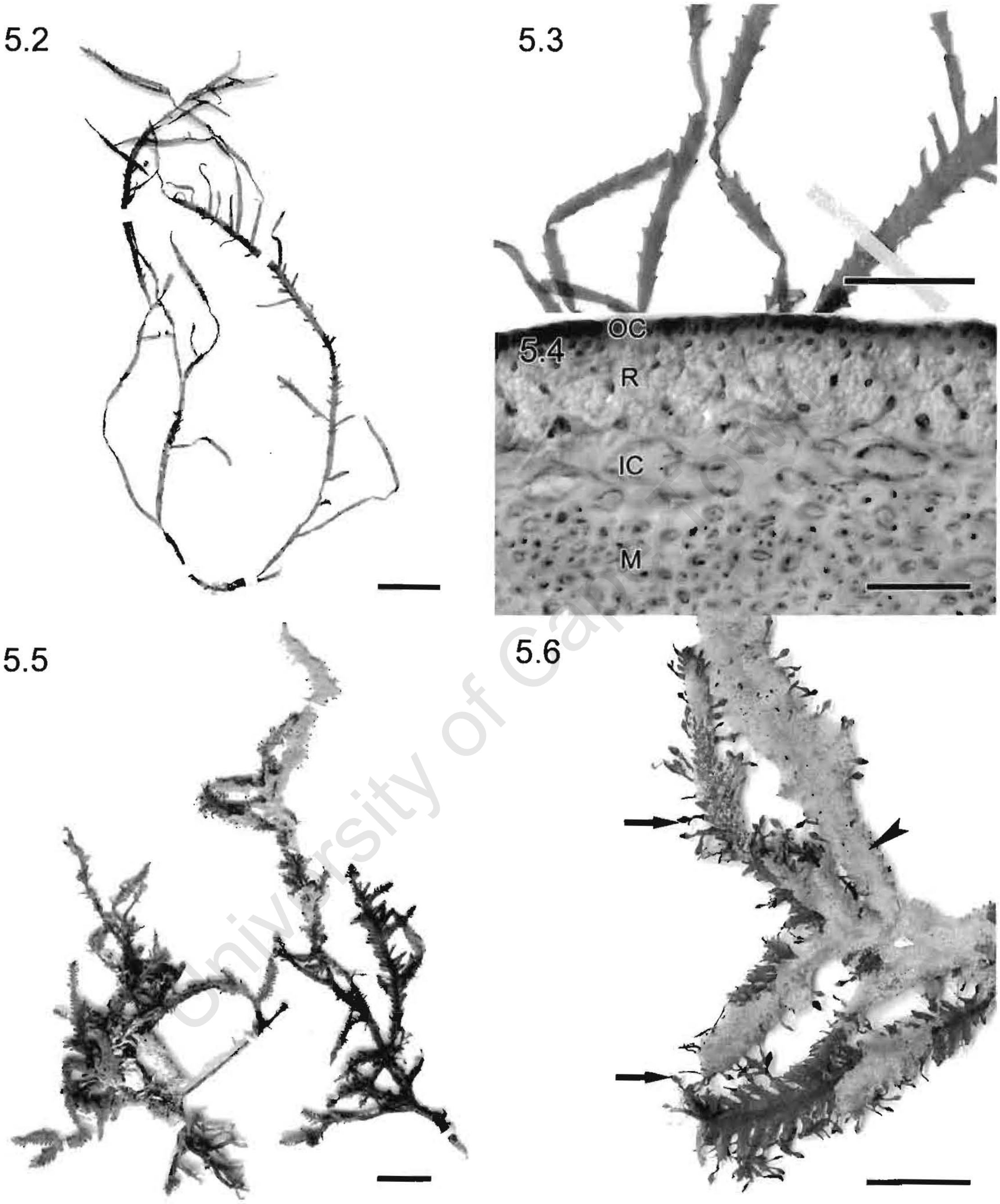


Fig. 5.2-5.4. *Ptilophora biserrata*. Fig. 5.2. Habit of isotype. Scale = 2 cm. Fig. 5.3. Axes and indeterminate laterals with serrate margins. Scale = 1 cm. Fig. 5.4. Transverse section of second order branch. Outer cortex (OC); rhizine layer (R); inner cortex (IC); medulla (M). Scale = 50 µm. Fig. 5.5-5.6. *Ptilophora coppejansii*. Fig. 5.5. Habit of holotype consisting of two fragments. Scale = 2 cm. Fig. 5.6. Closely spaced branches bearing cystocarps (arrow) on short lateral proliferations. The thallus is usually associated with a thickly encrusting sponge epiphyte (arrowhead). Scale = 1 cm.

2. *Ptilophora coppejansii* Tronchin. Type locality: Protea Banks Reef, KwaZulu-Natal Province, South Africa.

Fig. 5.5-5.6

Description

Plants up to 23 cm long. Fibrous holdfast of robust, terete haptera. Lower axis cylindrical, up to 6 mm in diameter. Up to four orders of branches, lower order branches 2-3 mm broad, pinnae narrower. Long major branches in distal parts with clearly visible midrib bearing thin, flexuous alae, regularly producing short (up to 3 mm long), closely spaced pinnae (up to 1.3 mm apart) and sparse, irregularly arranged indeterminate lateral branches which are in turn pinnate. In distal regions branches narrower than bearing branch. Numerous surface proliferations usually present proximally. Vegetative structure is four-layered. Cystocarps bilocular, borne terminally or subterminally on lateral proliferations. Spermatangia and tetrasporangia unknown.

Distribution

Only known from the type locality, Protea Banks Reef, KwaZulu-Natal Province, South Africa.

Specimens examined: Holotype - Protea Banks, Southern Pinnacle, -37 m; Freshwater; 4 ii 2001; 99070 (BOL). Protea Banks (KZN), Northern Pinnacle, -35 m; Anderson & Bolton; 29 vi 2000; KZN 1858 (GENT). Protea Banks, Salmon Bank, -27 m; Tronchin; 6 ii 2001; KZN 1992 (GENT) – wet preserved. Protea Banks, Southern Pinnacle, -34 m; Leliaert; 20 viii 1999; 511 (BOL) – wet preserved.

3. *Ptilophora diversifolia* (Suhr) Papenfuss in Botaniska Notiser: 214-216 (1940). Type locality: South Africa [between the Omtendo (Umtentu) River, Transkei and the Omsamculo (Umzimkulu) River, KwaZulu-Natal Province *fide* Drège, 155 (1843).

Fig. 5.7-5.14

Phyllophora diversifolia Suhr, 262 (1840)
Phyllophora spissa Suhr, 262-263 (1840)
Ptilophora spissa (Suhr) Kützing, 25 (1847)

Membranifolia diversifolia (Suhr) Kuntze, 903 (1891)

Description

Plants up to 30 cm long, holdfast fibrous and robust. Proximal axis diameter ca. 2.5 (-6) mm. Blades broadly alate (2-5 mm) wide with distinct midrib to apices. Branching to four orders, irregular and sparse. Distal branches elongate (up to 20 cm) and mostly unbranched with entire, undulate, crenate or eroded margins. Branch apices are rounded. Alae sometimes tapered proximally and often reduced to the midrib at the base of branches. Blades normally smooth but in aberrant morphs produce numerous surface proliferations predominantly from the midrib, very eroded margins, and may produce closely spaced (often abutted) lateral pinnae. Vegetative structure is four-layered. Cystocarps and tetrasporangial sori borne on short marginal and surface proliferations. Cystocarps bilocular. Spermatangia unknown.

Distribution

This species has been reported from the southern KwaZulu-Natal and Eastern Cape Provinces of South Africa (Drège 1831, Norris 1992b) from south of Durban to Port Elizabeth.

Specimens examined: Protea Banks, S. Pinnacle, -37m, KwaZulu-Natal, South Africa; Tronchin; 4 ii 2001; ET15 (BOL). Protea Banks, N. Edge, -33m, KwaZulu-Natal, South Africa; Tronchin; 5 ii 2001; ET17 & ET18 (BOL). Protea Banks, N. Pinnacle, South Africa, -37 m; De Clerck *et al.*; 5 ii 2001; KZN 1941 (GENT). Protea Banks, S. Pinnacle, South Africa, -37 m; De Clerck *et al.*; 4 ii 2001; KZN 1928 (GENT). Scottsburgh, South Africa, -35 m; De Clerck; 22 xii 1999; KZN 1420 (GENT). Widenham Natal South Coast; 14 ix 1981; R.N. Pienaar; 001525 (NU). Sea shore near Kei Mouth S. Africa; Flanagan; 1891; coll. no. 4 (35328, BOL). Lectotype (*Ptilophora spissa*) - prope Omsamculo; Drège; coll. no. 4143 (16292, W). Cap. b. Sp.; Suhr; 33301 (LD). In Africa Australi; Drège; s.n. (S).

Remarks

Ptilophora diversifolia has been found to produce thalli with an aberrant morphology and are often covered in sponge epiphyte. In such cases, blades produce numerous scale-like surface proliferations on the midrib and on the alae which may erode, leaving a series of parallel ridges on the alae adjacent to the blade margins, like the rungs of a ladder. The branching pattern of the seaweed is unchanged and thalli are still characterised by elongate and poorly branched distal branches [Fig. 10; also see specimen 1525 at NU and 35328 at BOL (Plate 35, Figs. a & b in Fan, 1961)]. Specimens exhibiting a second and rarely encountered type of aberrant morphology were recently discovered along the southern KwaZulu-Natal coast of South Africa. These plants are characterised by very dense surface proliferations, eroded branch margins and procurrent, pinnate branching on the elongate indeterminate branches (Fig. 5.8). The pinnae are irregularly arranged and variable in length and can be so closely spaced that their margins abut or overlap (Fig 5.13). Only the most distal regions of the thallus or young proximal branches have the smooth, undulate blades characteristic of *P. diversifolia*. The branch morphology (comprising dense surface proliferations and eroded margins), branch proportions and branching pattern of this aberrant form of *P. diversifolia* (Fig. 5.11 and 5.13) is practically identical to that of *P. spissa* (Figs. 5.12 and 5.14), a species only known until recently from the type specimen (Fig. 5.9), that was collected along the same stretch of coast in the early 1800's. Two additional specimens of *P. spissa* were located in the herbaria of Lund and Stockholm, but these specimens are more than likely isotypes if not fragments of the type specimen, since they have identical external morphological characteristics and are merely lateral branch fragments. A note by Norris on the specimen at Lund supports this opinion. *P. spissa* and *P. diversifolia* are believed to be conspecific. None of the specimens of *P. spissa* have smooth blades like those found in *P. diversifolia,* which is why the uncertainty about *P. spissa* has persisted for almost two centuries. Attempts were made to extract DNA from the lectotype specimen for *rbc*L gene sequencing, but were unsuccessful. Consequently, a molecular comparison with *P. diversifolia* cannot be carried out.

Since *P. spissa* and *P. diversifolia* were described simultaneously in the same publication (Suhr, 1840), neither name has priority according to the St. Louis Code of Botanical Nomenclature (Greuter *et al.* 2000). Having been repeatedly collected and being well known among phycologists, it seems appropriate to retain the name *P. diversifolia* and to propose *P. spissa* a synonym. The lectotype of *P. diversifolia* was destroyed when the Berlin Museum was bombed during World War II. The illustration of this specimen in

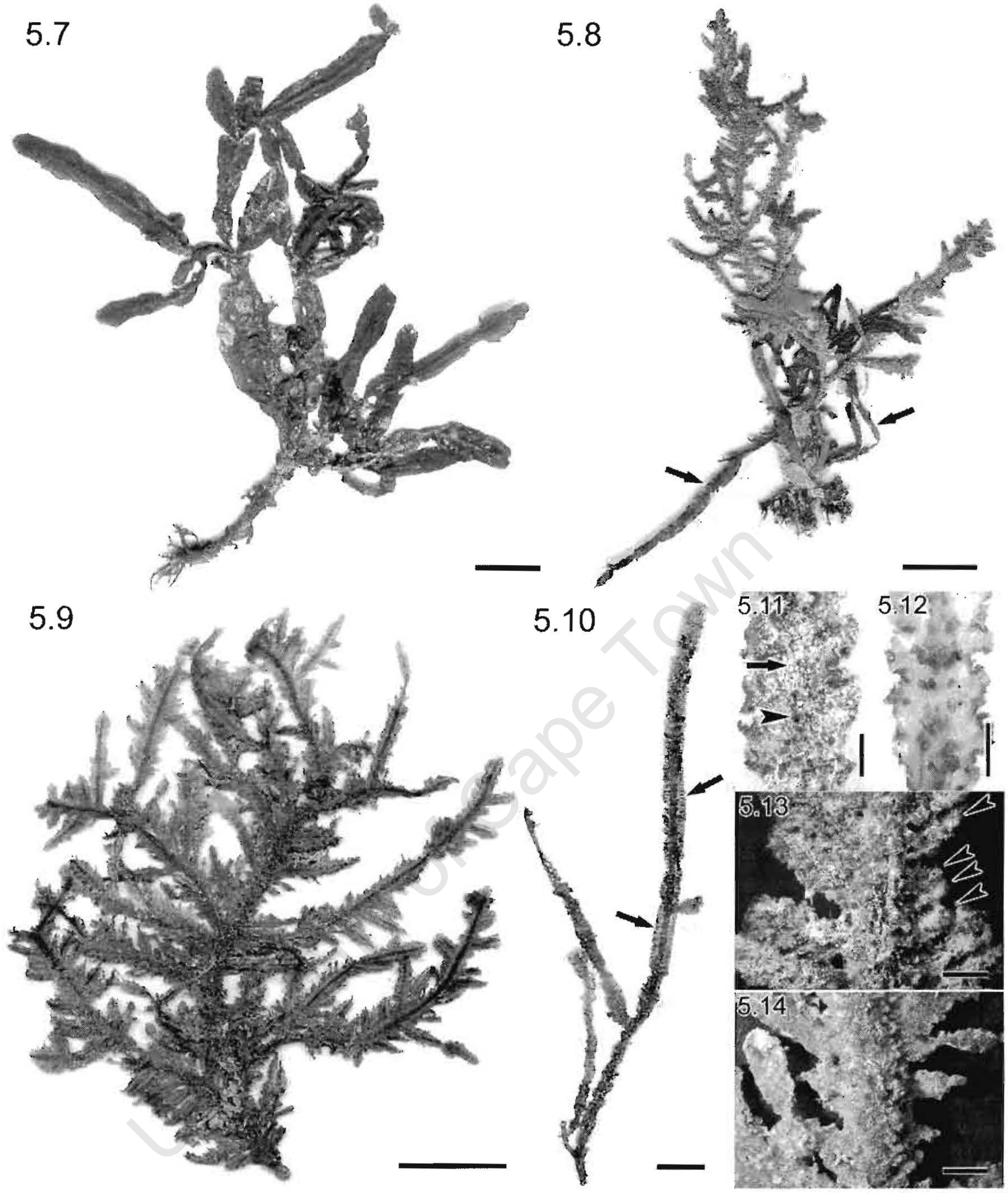


Fig. 5.7-5.14. *Ptilophora diversifolia* (and synonym *P. spissa*). Fig. 5.7. Habit of epitype. Scale = 1 cm. Fig. 5.8. Extremely proliferate habit of *P. spissa*-like specimen of *P. diversifolia*. Nonproliferate, unbranched blades are also produced which are generally free from sponge (arrow). Scale = 3 cm. Fig. 5.9. Habit of lectotype of *P. spissa*. Scale = 2 mm. Fig. 5.10. Fragment of *P. diversifolia* on the type-sheet of the *P. spissa* holotype specimen. The blade bears abundant surface proliferations arranged in places as a series of parallel ridges on the alae along the blade margins (arrow). Scale = 1 cm. Fig. 5.11. Surface view of a *P. diversifolia* blade covered in sponge epiphyte (white sponge spicule skeleton visible - arrow) and surface proliferations (arrowhead). Scale = 1 mm. Fig. 5.12. Surface view of proliferate blade of *P. spissa*. Scale = 1 mm. Fig. 5.13. *P. diversifolia* axis bearing procurrent pinnae (arrowhead) densely covered in surface proliferations and sponge epiphyte. Scale = 2 mm. Fig. 5.14. Pinnately branched axis of *P. spissa* densely covered in surface proliferations and sponge epiphyte. Scale = 2 mm.

Papenfuss (1940: 215, fig. 11) cannot be used unambiguously for the purpose of the precise application of the name since it does not clearly demonstrate some of the characteristic features of the species. The photograph does not show the alate and midribbed nature of the blade, nor the smooth, undulate, slightly crenate to eroded margins, due to the abundant surface and lateral proliferations it bears and the encrusting sponge epiphyte. Since specimens from the Drège collection were distributed to contemporary workers, the herbaria at Munich, Kiel, Lund and Stockholm were contacted in an attempt to locate potential lectotype specimens, but none could be found. Consequently, a specimen housed at BOL (ET18; Protea Banks, Northern Edge, -33m, KwaZulu-Natal, South Africa; Tronchin; 5 ii 2001) is designated as the epitype (Fig 5.7). This specimen was selected since it is an entire thallus and does not have either of the aforementioned aberrant morphologies. The frond clearly demonstrates the characteristic frond features discussed above of this species.

4. *Ptilophora helenae* (Dickinson) Norris in Botanica Marina 30: 256 (1987a). Type locality: Richards Bay, KwaZulu-Natal Province, South Africa.
Fig. 5.15-5.16

Gelidium helenae Dickinson, 1950: 565
Beckerella helenae (Dickinson) Fan & Papenfuss in Fan, 1961: 322

Description

Plants up to 40 cm. Frond compressed to flattened, midrib indistinct throughout. Axes mostly pectinate bearing procurrent, rostrate to acuminate determinate branches with irregularly disposed indeterminate branches. Up to four orders of closely spaced branches (ca. 1.3 mm between primary branches). The distance from the base of a primary branch to the point at which ramuli commence ranges from 0.7-7.7 mm (mean=2.6, std. dev.=0.97). Proximal axis margins bearing basal branch stubs. Vegetative structure is four-layered. Cystocarps and tetrasporangia borne terminally on pinnae which are sometimes abnormally extended, flattened and ligulate, conferring a feathered appearance to the thallus. Cystocarps bilocular. Spermatangia unknown.

Distribution

This species is known from drift collections at four locations spread along the coast of the northern KwaZulu-Natal Province of South Africa, but was only found attached at one location.

Specimens examined: Holotype – Natal, Richards Bay, South Africa; Rump; vii 1929; 561902 (BM). Mission Rocks (drift), KwaZulu-Natal Province, South Africa; Bolton; 10 viii 2002; 99071 (BOL). Mission Rocks, infralittoral fringe; De Clerck & Leliaert; 13 vi 2003; KZN 2229 (GENT/BOL).Richards Bay (drift), South Africa; Pocock; 17 x 1951; 9633 (GRA). Richard's Bay (drift), South Africa; Pocock; 24 xi 1962; 13241 (GRA). Richard's Bay (drift), South Africa; Pocock; 17 xi 1951; 7103 (GRA).

5. *Ptilophora hildebrandtii* (Hauck) Norris in South African Journal of Botany 56,1: 133-134 (1990b). Type locality: Mombasa, Kenya.
Fig. 5.17-5.18

Thysanocladia hildebrandtii Hauck, 217-218 (1886)
Gelidium hildebrandtii (Hauck) Schmitz, 196-197 (1894)
Beckerella hildebrandtii (Hauck) Kylin, 139 (1956)

Description

Plants up to 40 cm long developing from a fibrous holdfast producing flattened, elongate (up to 7 cm) and straggly rhizoids. Midrib indistinct throughout. Frond compound pinnate with up to six orders of evenly spaced branches. Primary branches on average 0.7 mm apart (std. dev.=0.24). Branches are subtended at broad angles (50-80°). Ramuli commence at a uniform distance (1-2 mm) from the base of the bearing branch. Branches flat, uniform in width, always narrower than bearing branch. Proximal axis margins often bear basal branch stubs. Vegetative structure is four-layered. Only sterile plants known.

Distribution

Reported from Kenya, Tanzania, Zanzibar (Hauck 1886, Lawson 1980) and the northern KwaZulu Natal Province of South Africa (Norris, 1990b), and is presumed to have a tropical East African distribution (Norris, 1992b). *P. hildebrandtii* has also been reported

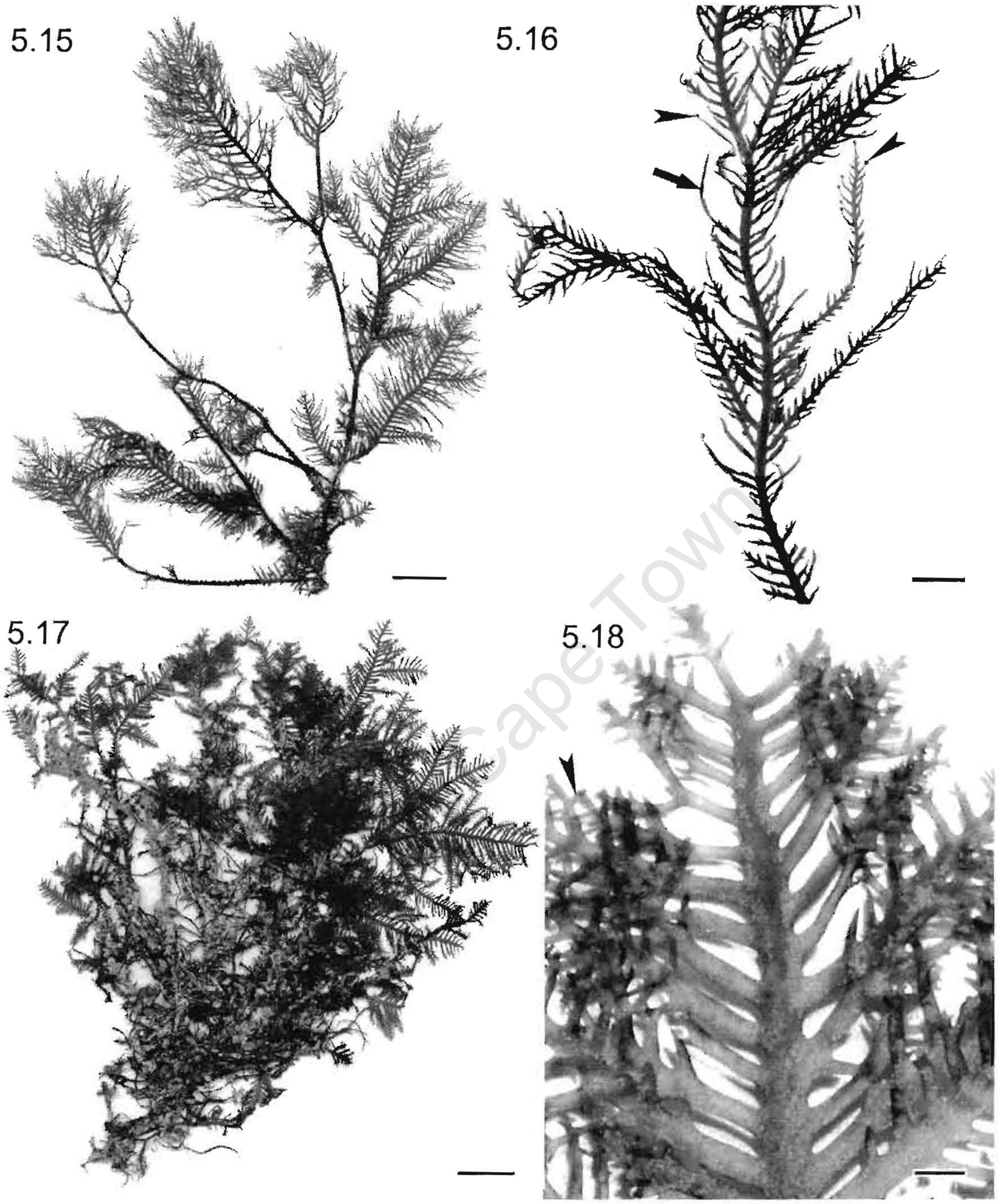


Fig. 5.15-5.16. *Ptilophora helenae*. Fig. 5.15. Habit of plant bearing unusually elongate, ligulate pinnae conferring a feathered appearance to the plant. Scale = 2 cm. Fig. 5.16. Pectinately branched frond with evenly spaced rostrate pinnae, some considerably longer and ligulate in shape (arrow). Cystocarps borne terminally on short or elongate pinnae (arrowhead). Scale = 1 cm. Fig. 5.17-5.18. *Ptilophora hildebrandtii*. Fig. 5.17. Densely branched, crowded habit. Scale = 2 cm. Fig. 5.18. Uniformly spaced, pinnate branching showing narrow (ca. 0.1 mm) 6th order branches (arrowhead). Ramuli issue at a uniform distance from the base of the bearing branch. Scale = 1 mm.

from Madagascar by Farghaly (1980); however, specimens were not collected by the author himself and the original report cannot be traced. This report is therefore questionable.

Specimens examined: Lectotype – Mombasa, Kenya; Hildebrandt; vii 1876; s.n. (L). Tiger Reef (offshore from Kosi Bay), KwaZulu-Natal, South Africa; Tronchin; 13 viii 1999; KZN 520 (GENT/BOL). Sodwana Bay, 5 Mile Reef, 18-22 m, North Coast, Natal, R.S.A; Sydenham; 14 v 1986; NAT5285 (NU). Kenya, Mombasa, Mc. Kenzie Pt., -3 m; Coppejans & Buckman; 21 i 1986; HEC 5930 (GENT). Kenya, Mombasa, Mc. Kenzie Pt., -4 m; Coppejans; 2 vii 1985; HEC 5560 (GENT). Msambweni (Kenya), -30 m; Coppejans; 22 ix 1991; HEC 8825 (GENT).

6. *Ptilophora irregularis* (Akatsuka & Masaki) Norris in Botanica Marina 30: 256 (1987a). Type locality: Kôzu-sima, Izu-shotô archipelago, Japan.

Beckerella irregularis Akatsuka & Masaki, 11-13 (1983)

Description

Plants to 64 cm long. Axes more or less uniform in width (3.1-4.1 mm), midrib indistinct throughout. Up to three orders of branching, branch length and width variable, basally constricted, margins smooth. Ultimate pinnules distichous, up to 10mm long, spaced sparsely and irregularly at intervals of 2-63 mm. Vegetative structure is four-layered, medullary filaments not continuous between main axis and lateral branches. Tetrasporangial sori borne terminally and subterminally on filiform pinnules (to 25 mm long) and often bear constrictions. Tetrasporangia cruciate. Sexual plants unknown.

Distribution

Known from central Japan along the coast of the Kanagawa Prefecture. It has also been reported from the nearby Kôzu-sima and Izu-ôsima islands, and the Izu-shotô archipelago (Akatsuka and Masaki 1983, Akatsuka 1987).

Remarks

According to Akatsuka and Masaki (1983), *P. irregularis* produces a creeping axis, a feature absent in all other *Ptilophora* species. Since specimens of this species were unavailable, this cannot be confirmed.

7. *Ptilophora leliaertii* Tronchin. Type locality: Protea Banks Reef, KwaZulu-Natal Province, South Africa.
Fig. 5.19-5.20

Description

Plants up to 35 cm long. Fibrous holdfast of robust, terete haptera. Lower axis cylindrical (ca. 2 cm in diameter) becoming narrowly alate in lower branches and flattened and consistently concave in the pinnate reaches with midrib less distinct. Up to six orders of regular distichous, compound pinnate branches mostly 1.5-2 mm broad and fairly uniformly spaced, 1-2 mm apart. In flattened regions, branches, except ultimate pinnae, are generally broader (up to 30%) than their bearing branch, though sometimes uniform in width, and are roughly as wide at their base as the bearing branch. Pinnae are linear with acute to obtuse apices. Vegetative structure is four-layered. Fertile structures borne terminally or subterminally on lateral branchlets. Cystocarps bilocular, ostiole sometimes lipped. Tetrasporangial and spermatangial sori elliptical, tetraspores cruciate, spermatangial mother cells forming single-layer outer cortex.

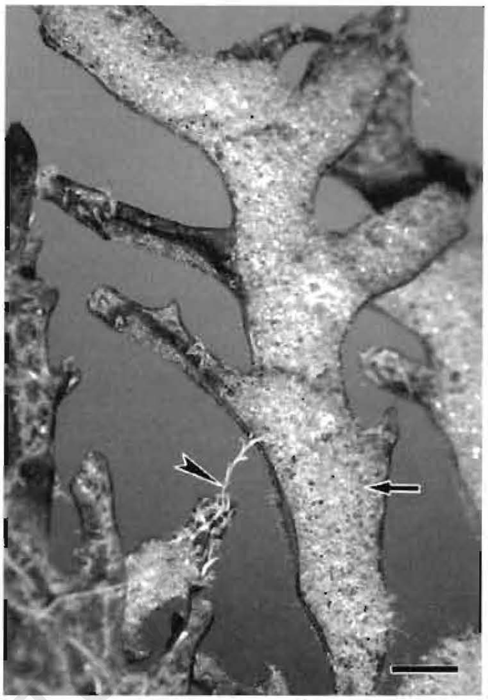
Distribution

Only known from the type locality, Protea Banks Reef, KwaZulu-Natal Province, South Africa, and from drift on a nearby beach.

Specimens examined: Holotype - Protea Banks, Southern Pinnacle, -37 m; Tronchin; 4 ii 2001; 99068 (BOL). Protea Banks (KZN), Southern Pinnacle, -34 m; Leliaert *et al.*; 20 viii 1999; KZN 881 (GENT, BOL). Protea Banks, Northern Pinnacle, -35 m; Anderson and Bolton; 29 vi 2000; KZN 1857 (GENT). Location unknown; Simons; 1966; 109599 (BOL). Protea Banks, Southern Pinnacle, -34m; Leliaert; 20 viii 1999; 512 (BOL) - wet preserved. Palm Beach (KZN) - drift; Hommersand; 23 vii 1993; s.n. Uvongo Rocks (drift); Pocock; 1 xi 1951; 10090 (GRA).

5.19

5.20



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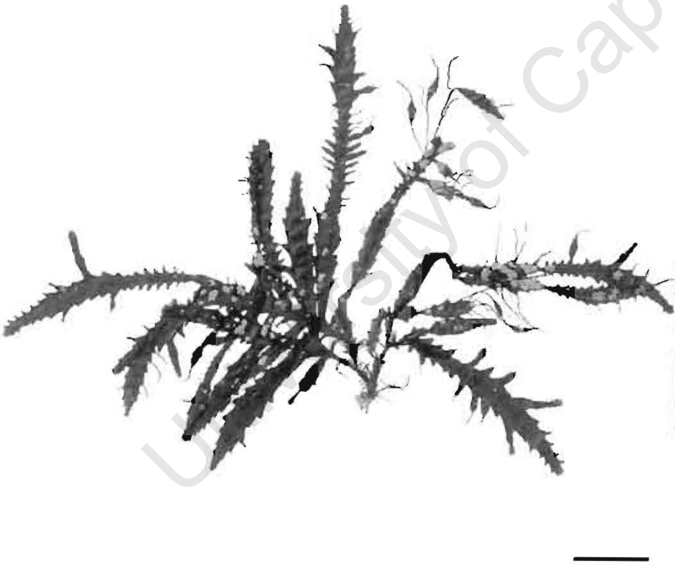

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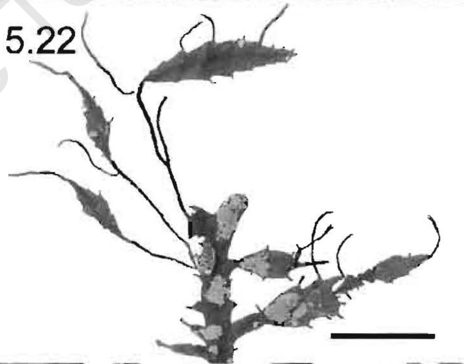

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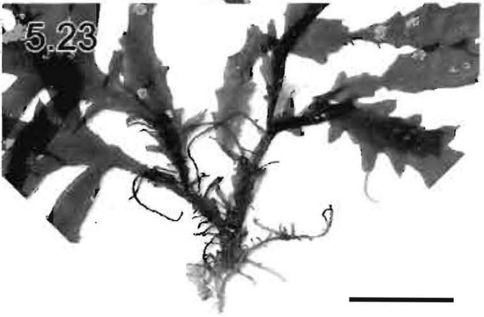


Fig. 5.19-5.20. *Ptilophora leliaertii*. Fig. 5.19. Habit of plant, covered in places by sponge (arrow) with crustose coralline (arrowhead) enveloping the axes. Scale = 2 cm. Fig. 5.20. Concave distal flattened branches with a sponge (arrow) and hydroid (arrowhead) nestled within the concavity, some pinnae bearing in-rolled margins. Scale = 1 mm. Fig. 5.21-5.23. *Ptilophora mediterranea*. Fig. 5.21. Habit of isotype. Scale = 2 cm. Fig. 5.22. Terminal adventitious rhizoids that may give rise to new blades or entire individuals. Scale = 1 cm. Fig. 5.23. The holdfast which is relatively insignificant compared to other *Ptilophora* species. Scale = 1 cm.

8. *Ptilophora mediterranea* (H. Huvé) Norris in Botanica Marina 30: 258 (1987a). Type locality: S. Peloponnesus (west of Cape Matapan), Greece.
Fig. 5.21-5.23

Phyllophora aegei Giaccone, 405-406 (1968)
Beckerella mediterranea Huvé, 32-52 (1962)

Description

Plants up to 11 cm long, attached by relatively insignificant holdfast, rhizoids ≤0.5 mm in diameter. Midrib distinct throughout. Major branches lanceolate, pinnately branched to four orders with sparse, irregularly disposed indeterminate branches and regularly, suboppositely arranged pinnules shaped like blunt or mucronate serrations. Up to 6 mm between pinnae, this distance being greatest along central portion of bearing branch. Branches always narrower than bearing branch, 1-5 mm wide. Indeterminate branch apex broadly rounded. Adventitious rhizoids frequently produced from apices of branches and serrulae which can give rise to new blades or individual thalli. Vegetative structure is four-layered. Tetrasporangial branchlets slightly swollen and variously lobed, sori without sterile margins. Cystocarps and spermatangia unknown.

Distribution

This species is widespread in the Aegean Sea, reported from numerous Greek islands including Crete, Peloponnesus, Ceos, Thera, Kea, Santorini, Dia and Zea (Huvé 1962, Giaccone 1968), as well as from Cyprus (Cabioc'h *et. al.*, 1992b).

Specimens examined: Isotype - Sud du Péloponèse, face ouest du Cap Matapan, –25 m, Campagne CALYPSO; Huvé; 11 ix 1955; 1218 (PC).

9. *Ptilophora pectinata* (A. & E.S. Gepp) Norris in Botanica Marina 30: 252 (1987a). Type locality: Maroubra Bay, New South Wales, Australia.
Fig. 5.24-5.25

Pterocladia lucida (R. Brown ex Turner) J. Agardh forma *pectinata* A. & E.S. Gepp, 254 (1906)

Pterocladia pectinata (A & E.S. Gepp) Lucas, 408 (1931)
Gelidium lucasii May, 226 (1944)
Beckerella pectinata (A. Gepp & E. Gepp) Fan & Papenfuss in Fan, 322 (1961)

Description

Plants up to 30 cm long. Frond compressed to flattened, midrib indistinct throughout. Up to four orders of branches, axes mostly pectinate bearing procurrent, rostrate to acuminate determinate branches with irregularly disposed indeterminate branches. Branches closely spaced (up to 3 mm apart). The distance from the base of a primary branch to the point at which ramuli commence ranges from 1.4-10.3 mm (mean=5.2, std. dev.=1.57). Proximal axis margins bearing basal branch stubs. Vegetative structure is four-layered. Cystocarps and tetrasporangia borne terminally on pinnae, or on flattened, filiform ligules (up to 3 mm long) borne from distal ends of pinnae. Cystocarps bilocular. Spermatangia unknown.

Distribution

This species has been reported from North Island, New Zealand: from North Cape to the Coromandel Peninsula (Adams, 1994) and from East Cape (May, 1944). It has also been reported from various locations along the coastline of New South Wales (May 1944, Akatsuka 1987, Fan 1961, Millar 1990) and Queensland, Australia (Millar, 1990). Known mostly from drift collections.

Specimens examined: Holotype - Maroubra Bay, New South Wales (Aust.); Lucas; vii 1901; coll. no. 9 (612225, BM). Richmond River Entrance, New South Wales (Aust.); 32659 (LD – Herb. Agardh). Coach near Mt. Dromedary (Aust.); 32658 (LD – Herb. Agardh).

10. *Ptilophora pinnatifida* J. Agardh in Lunds Universitets Årsskrift, Afdeliningen för Mathematik och Naturvetenskap 21,8: 79 (1885). Type locality: Cape Colony, Algoa Bay, South Africa.
Fig. 5.26-5.28

Gelidium pinnatifidum (J. Agardh) Schmitz, 194 (1894)

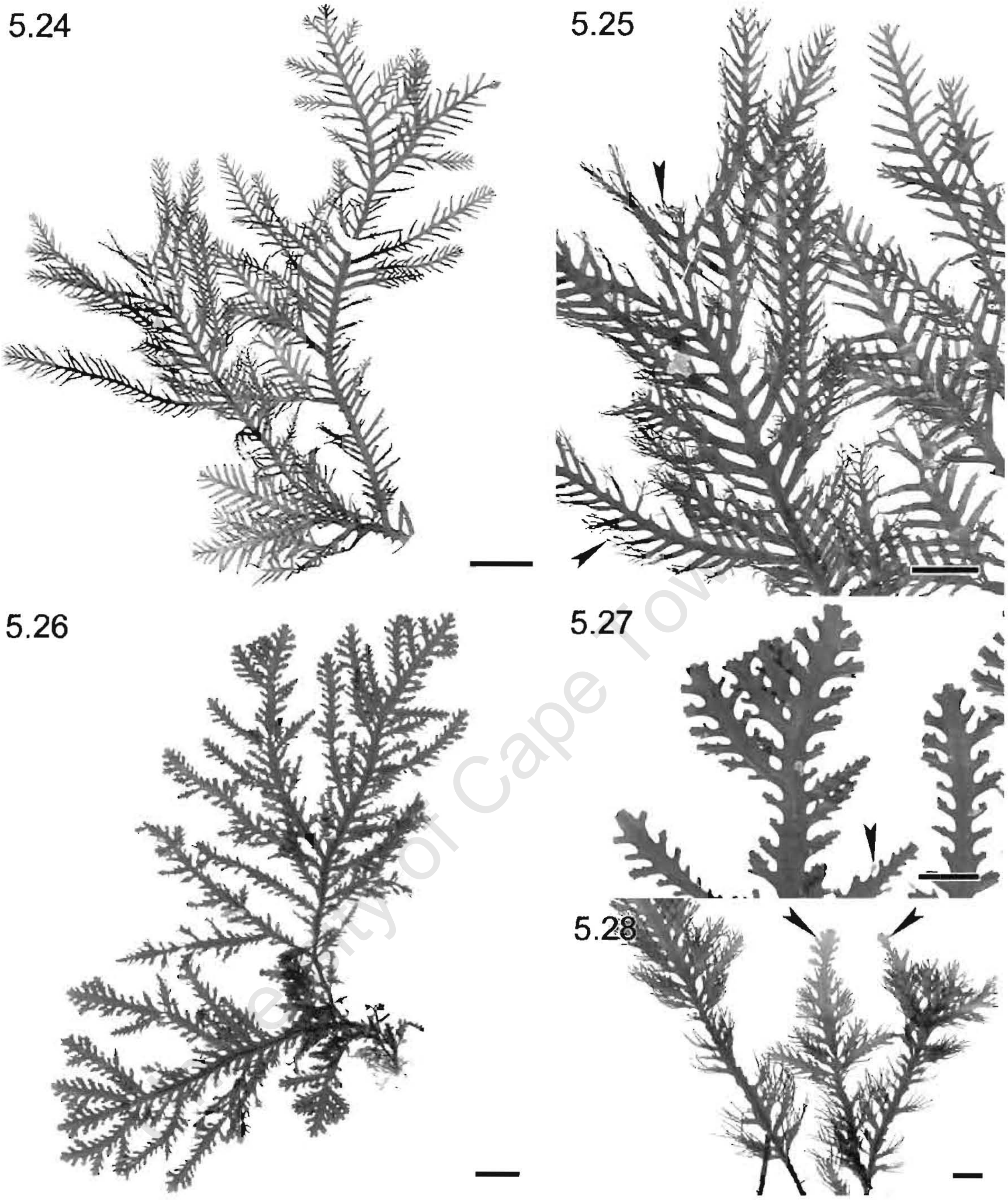


Fig. 5.24-5.25. *Ptilophora pectinata*. Fig. 5.24. Habit of cystocarpic plant. Scale = 2 cm. Fig. 5.25. Rostrate, evenly-spaced pinnae. Cystocarps borne apically on filiform branchlets borne terminally on pinnae. (arrowhead). Scale = 1 cm. Fig. 5.26-5.28. *Ptilophora pinnatifida*. Fig. 5.26. Habit. Scale = 2 cm. Fig. 5.27. Distal pinnatifid branches. Pinnae are triangular to linear to cuneate with blunt to tridentate apices. Tetrasporangial proliferations borne on pinnule apices (arrowhead). Scale = 1 cm. Fig. 5.28. Cystocarpic thallus bearing elongate, flattened ligules from the apices of pinnae. Cystocarps borne terminally or subterminally on the ligules. Ligules are shortest on newly developed distal branches. Indeterminate branch ends may appear quercifoliate due to initial regrowth after injury (arrowhead). Scale = 1 cm.

Beckerella pinnatifida (J. Agardh) Kylin, 139 (1956)
Ptilophora beckeri Holmes, 350 (1896)
Beckerella beckeri (Holmes) Kylin, 139 (1956)

Description

Plants up to 35 cm long. Proximal axis cylindrical becoming flattened distally with distinct midrib throughout except in ultimate and penultimate pinnae. Branching compound pinnate to five orders. Distal branches are pinnatifid, up to 5 mm wide, pinnae ca. 2 (-5) mm apart. Ultimate pinnae are triangular to linear to cuneate in shape, procurrently curved, with truncate to tridentate apices. Indeterminate branch ends sometimes quercifoliate, apices truncate to tridentate. Vegetative structure four-layered. Tetrasporangia, cystocarps (bilocular) and spermatangia borne apically and marginally on ultimate pinnae. Tetrasporangial and spermatangial sori borne on short surface proliferations as well. Cystocarpic proliferations sometimes elongate and ligulate, usually <6 mm long, becoming fertile terminally or subterminally.

Distribution

This species is known from the southern KwaZulu-Natal and Eastern Cape Provinces of South Africa (Norris, 1992b). The holotype is from the Cape Colony, Algoa Bay (Port Elizabeth). Agardh (1885), in his original description, stated that this species *apparently* occurred at the Cape of Good Hope and to a lesser extent at Algoa Bay. However, *P. pinnatifida* has not been collected since south of Algoa Bay.

Specimens examined: P. pinnatifida: Holotype – Algoa Bay, Cape Colony; Holub; v 1883; 33299 (LD). Protea Banks, KwaZulu-Natal, South Africa; Tronchin; viii 1999; KZN 808 (BOL/GENT). Broken Reef, Trafalgar (South Africa), 25-28 m; Coppejans *et al.*; 21 viii 1999; KZN 948 (GENT). The Kowie, South Africa; Becker; 30 vii 1892; 109597 (BOL). The Kowie, South Africa; Becker; 28 vi 1893; 109598 (BOL). *P. beckeri*: Fragment and photoprint of type - South Africa, The Kowie; Becker; 30 vii 1892; 561901 (BM).

Remarks

Plants usually produce fertile ligules that are 1-6 mm long and elongate fertile ligules up to 2 cm in length are rarely produced (Fig. 5.28). Elongate ligules are partly what led

Holmes (1896) to describe a separate species, *Ptilophora beckeri*, which was later placed in synonymy with *Ptilophora pinnatifida* (as *Beckerella pinnatifida*) by Akatsuka (1987). These structures have only been found in cystocarpic plants. The type specimen of *P. beckeri* in BM (561901), and a similar specimen in BOL (109597) also collected by Becker (Fig. 5.28), show a range in ligule length from 1 mm (in most distal regions of the thallus) to 2 cm (in proximal regions), with the majority tending to be about 1-1.2 cm long. Since short ligules are found distally and long ligules found proximally on the thallus, there seems to be a developmental sequence which could relate to the perennial nature of the plant. It is possible that in some cases, ligules are produced but do not become fertile until the following growing season when their length is augmented and new ligules are produced in distal regions.

11. *Ptilophora prolifera* (Harvey) J. Agardh in Species Genera et Ordines Algarum... Volumen Tertium: de Florideis Curae Posteriores. Part 1. 555 (1876). Type locality: Fremantle, Western Australia.
Fig. 5.29-5.31

Gelidium proliferum Harvey, 551 (1855)

Description

Plants up to 45 cm long. Holdfast and proximal axis robust, the latter being usually 4 (-7) mm in diameter. Midrib generally indistinct but may be visible in older parts. Up to four orders of compound pinnate branching. In flattened distal regions, branches always narrower than bearing branch and may be concave, pinnules normally not concave. Branch width 0.5-6 mm, up to 4 mm between branches. Pinnae are simple, procurrently curved, tapering slightly to rounded apices. Normally producing abundant short, simple to pinnately branched surface proliferations. Vegetative structure is four-layered. Short cystocarpic branchlets borne apically or laterally on pinnae and from the frond surface in distal regions, cystocarp bilocular. Spermatangial and tetrasporangial plants unknown.

Distribution

Reported from Western Australia (Womersley and Guiry 1994, Huisman 2000, Kützing 1869): Geraldton to Albany, including Penguin Island and Rottnest Island.

Specimens examined: Isotype – Fremantle, Nov. Hollandia Occident. (Aust.); Harvey; 33306 (LD - Herb. Agardh). Bunbury, Nov. Hol. Occid. (W. Aust.) ; 33302 (LD – Herb. Agardh). Roe Reef, Rottnest Island, W. Aust., 42-52ft; Kraft & Saunders; 2 ii 1994; K9683 (Herb. Kraft). Pt. Clune, Rottnest Island, W.Aust., 43’ on patch reef; Kraft & Millar; 6 xii 1984; 38184 (MELU-A). Penguin Island (Aust.), 7-8 m; Kraft & Huisman; 6 ix 1990; K8460 (Herb. Kraft). Geraldton, W. Aust., drift at Jarrah St.; G. & R. Kraft; 8 x 1990; K9989 (Herb. Kraft). Penguin Is. (Aust.), 7-8 m; Kraft & Huisman; 6 ix 1990; K8460 (Herb. Kraft). Geraldton, W. Aust., drift at Jarrah St.; G. & R. Kraft; 9 x 1990; K9971 (Herb. Kraft). Yanchep (Aust.), drift; Kraft & Herrington; 5 ix 1990; K8437 (Herb. Kraft). Cottesloe (Aust.), drift; Smith; viii 1964; s.n. (Herb. Kraft).

12. ***Ptilophora pterocladioides*** Andriamampandry in Cryptogamie Algologie 9: 244-247 (1988). Type locality: Mokala, near Fort-Dauphin (Taolanaro), Madagascar.

Fig. 5.32- 5.36

Beckerella pterocladioides (Andriamampandry) P. Silva, 132 (1996)

Description

Plants up to 25 cm long. Frond flattened and flimsy, irregularly and sparsely branched to six orders. Branches basally constricted, 0.5-2 mm wide, midrib only visible in proximal parts. Margins smooth with frequent, irregularly disposed constrictions. Ultimate branchlets generally oblanceolate with rounded apices. Vegetative structure is four-layered. Spermatangia develop in terminal swellings of ultimate branchlets. Cystocarps and tetrasporangia unknown.

Distribution

Only known from the type locality, Fort Dauphin, Mokala, Madagascar (Andriamampandry, 1988).

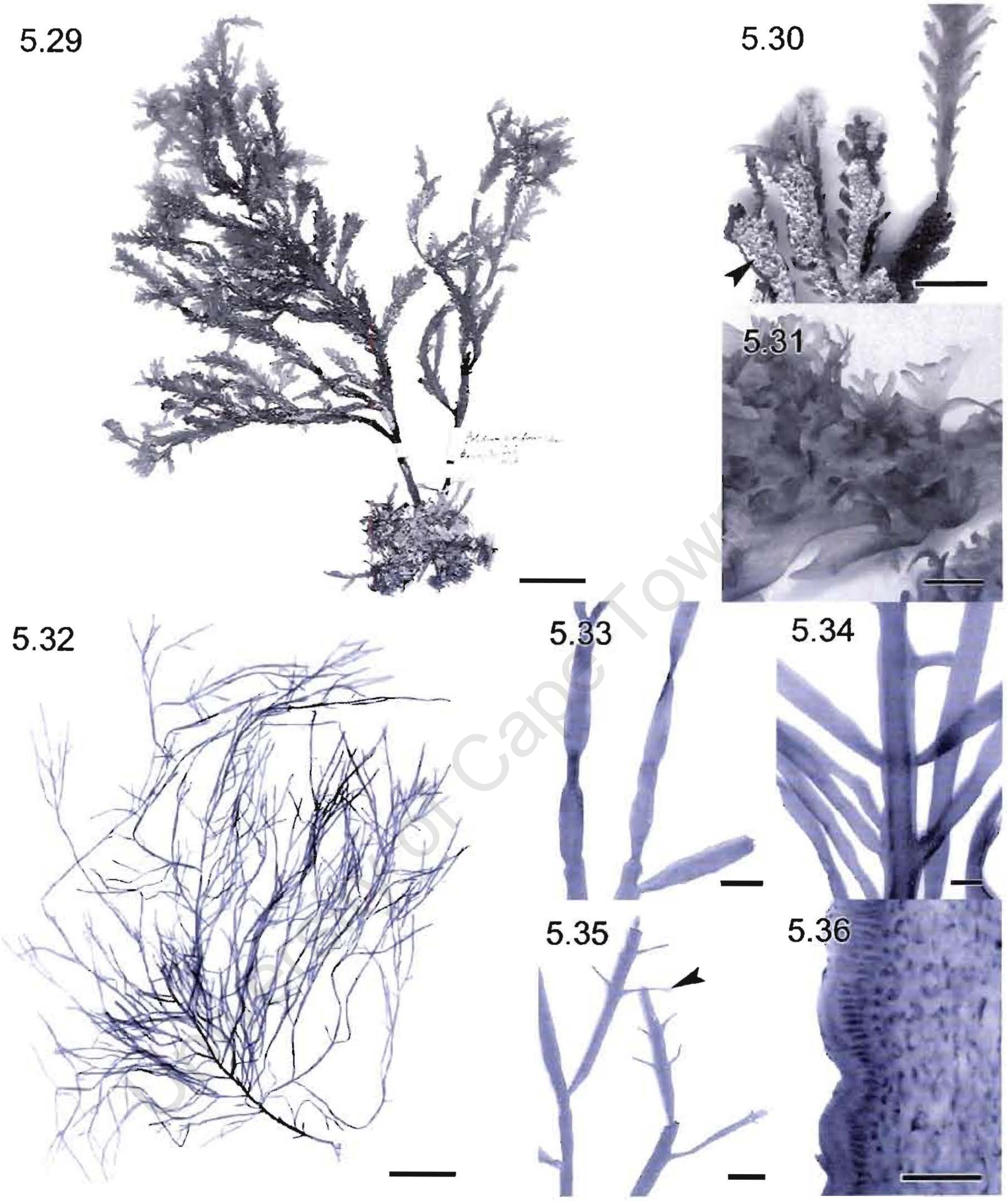


Fig. 5.29-5.31. *Ptilophora prolifera*. Fig. 5.29. Habit of isotype. Scale = 3 cm. Fig. 5.30. Distal procurrently pinnate branches lacking midribs. Sponge epiphyte (white; arrowhead) is engrained between the surface proliferations in places. Scale = 1 cm. Fig. 5.31. Pinnately branched proliferations developed from the surface of a distal flattened blade. Scale = 1 mm. Fig. 5.32- 7.36. *Ptilophora pterocladioides*. Fig. 5.32. Habit of holotype. Scale = 3 cm. Fig. 5.33. Irregularly disposed branch constrictions. Scale = 1 mm. Fig. 5.34. Midrib visible in proximal main axis. Scale = 1 mm. Fig. 5.35. Spermatangial sorus borne terminally on ultimate pinnule. Scale = 2 mm. Fig. 5.36. Transverse section of spermatangial sorus with elongate spermatangial mother cells forming a single-layered outer cortex . Scale = 25 µm.

Specimens examined: Holotype - Fort. Dauphin, Mokala (Madagascar); Decary; 23 vi 1962; 2706 (PC). Paratype - Fort Dauphin (Madagascar); Crosnier; v 1960; 5162 (PC).

Remarks

Andriamampandry (1988) noted the presence of fertile structures in the type material stating that they were probably tetrasporic but had not yet differentiated. One pinnule bearing a fertile structure was found on the holotype specimen (Fig. 5.35). Transverse sections of this structure revealed a single-layered outer cortex of elongate spermatangial mother cells (Fig. 5.36).

13. *Ptilophora rhodoptera* Norris in Botanica Marina 30: 254 (1987a). Type locality: Rocky Bay, Park Rynie, Natal, South Africa.
Fig. 5.37-5.38

Description

Plants up to 35 cm long attached by fibrous, robust holdfast. Main axis basally cylindrical, 3.5 mm in diameter. Branching to three orders, irregular, sparse, often with adventitious indeterminate branching from the blade surface. Broadly alate blades (up to 1 cm) with distinct midrib to branch apices. Blades with undulate margins bearing blunt or mucronate serrations (due to apical papillae). Alae taper to midrib basally on branches often appearing petiolate appearance as a result. Apices are rounded, retuse to emarginated, often with a small bladelet in the apical incision. Vegetative structure is four-layered. Tetrasporangial branchlets cordate, often retuse with a narrow sterile margin. Sexual plants unknown.

Distribution

Known only from the southern KwaZulu-Natal Province of South Africa.

Specimens examined: Holotype - Rocky Bay, Park Rynie; Pienaar; 4 x 1982; NAT370 / 9522 (NU). Shelly Beach, Salmon Banks, South Africa, -27 m; De Clerck *et al.*; 6 ii 2001; KZN 1991a (GENT).

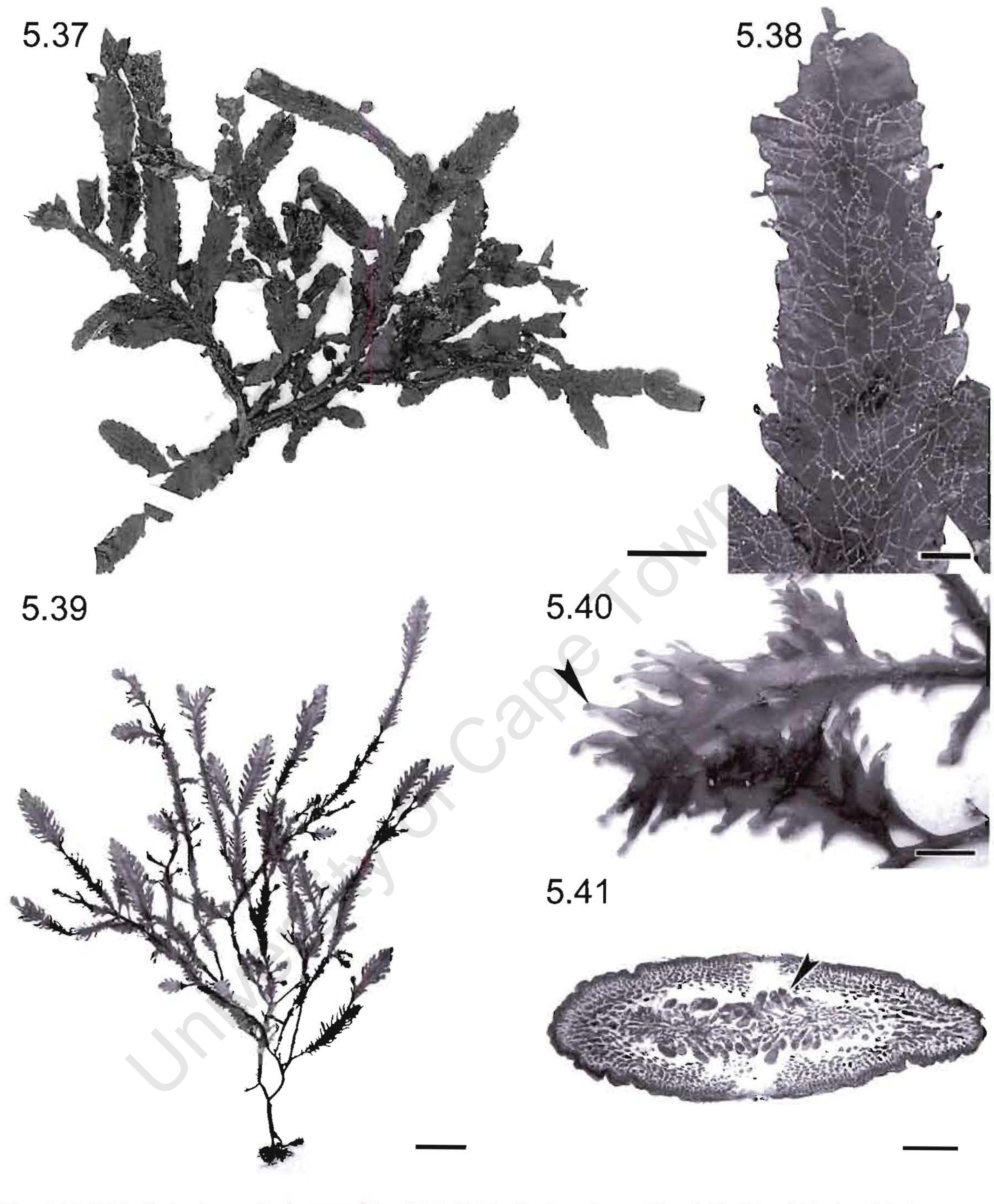


Fig. 5.37-5.38. *Ptilophora rhodoptera*. Fig. 5.37. Habit. Scale = 2 cm. Fig. 5.38. Distal blade with the prostrate system of a hydroid covering the surface in a network of tubes. The margins are crenate to serrate, some serrations appearing mucronate due to apical papillae that develop into small bladelets that may become fertile. Scale = 0.25 mm. Fig. 5.39-5.41. *Ptilophora rumpii*. Fig. 5.39. Habit. Scale = 2 cm. Fig. 5.40. Cystocarps borne apically on rostrate pinnae. Scale = 4 mm. Fig. 5.41. Transverse section of bilocular cystocarp with one ostiole per locule. Carposporangia (arrowhead) develop either side of a central placental mass. Scale = 50 μm

14. ***Ptilophora rumpii*** (Dickinson) Norris in Botanica Marina 30: 254-256 (1987a). Type locality: Richards Bay, KwaZulu-Natal Province, South Africa.
Fig. 5.39-5.41

Gelidium rumpii Dickinson, 565 (1950)
Beckerella rumpii (Dickinson) Papenfuss & Fan in Fan, 322 (1961)

Diagnosis

Plants up to 35cm tall. Midrib distinct in flattened parts, frond alate in sections, alae tapering at either end to midrib. Up to four orders of branches, 1-5 mm wide. Major branching irregular and sparse, regularly pinnate in alate sections. Margins smooth to eroded, alae reduced to midrib proximally and in places distally. Ultimate pinnae triangular to linear when young, rostrate and procurrently curved when mature with acute to obtuse apices. Indeterminate branch apex obtuse to tridentate. Vegetative structure is four-layered. Tetrasporangia borne terminally on short lateral and surface proliferations. Cystocarps borne terminally on ultimate pinnae or lateral proliferations. Spermatangia unknown.

Distribution

The only confirmed reports of *P. rumpii* are from the type locality, Richard's Bay, Kwa-Zulu Natal Province of South Africa. All specimens were collected from drift and probably originated from deep subtidal environments. *P. rumpii* has also been reported from Kenya in various species check lists (Isaac 1971, Lawson 1980, Moorjani 1976) without illustrations or descriptions being provided; consequently, these reports cannot be confirmed.

Specimens examined: Holotype – Natal, Richards Bay, South Africa; Rump; vii 1929: 561908 (BM). Richard's Bay (drift), South Africa; Pocock; 24 xi 1962; 13242 (GRA). Richard's Bay (drift), South Africa; Pocock; 17 x 1951; 9640 (GRA). Richard's Bay (drift), South Africa; Pocock; 17 x 1951; 7102 (GRA).

15. *Ptilophora scalaramosa* (Kraft) Norris in Botanica Marina 30: 258 (1987a). Type locality: Bulusan, Sorsogon Province, Luzon, Philippine Islands.
Fig. 5.42-5.43

Beckerella scalaramosa Kraft, 85-91 (1976)

Description

Plants up to 30 cm long. Fibrous holdfast of flattened, elongate (5-6 cm) rhizoids. Midrib only in lower parts. Frond compound pinnate with up to four orders of branches that are uniformly spaced, on average 1 mm apart. Ramuli commence at very uniform distance (2-3 mm) from base of bearing branch. Branches flat, linear, always narrower than bearing branch. Proximal axis margins notched (basal branch stubs). Vegetative structure is four-layered. Cystocarps bilocular, borne subterminally on pinnae. Tetrasporangia cruciate, borne terminally or in patches within flat surfaces of pinnae. Spermatangia unknown.

Distribution

Reported from Sorsogon Province, Luzon Island, in the Philippines (Kraft 1976, Kraft *et. al.* 1999), Java in Indonesia (Akatsuka, 1987) and Queensland in Australia (Phillips, 1997).

Specimens examined: Isotype – Bulusan, Sorsogon Prov., Luzon, Philippines, 3-4 m; Kraft; 22 iii 1968; G.K. 431 (Herb. Kraft). Bulusan, Sorsogon Prov., Luzon, Philippines, drift; Kraft; 7 iii 1968; G.K. 115 (Herb. Kraft).

16. *Ptilophora subcostata* (Okamura ex Schmitz) Norris in Botanica Marina 30: 258 (1987a). Type locality: Mie and Kanagawa Prefectures, Japan.
Fig. 5.44-5.45

Gelidium subcostatum Okamura ex Schmitz, 190-201 (1894)
Beckerella subcostata (Okamura ex Schmitz) Kylin, 139 (1956)

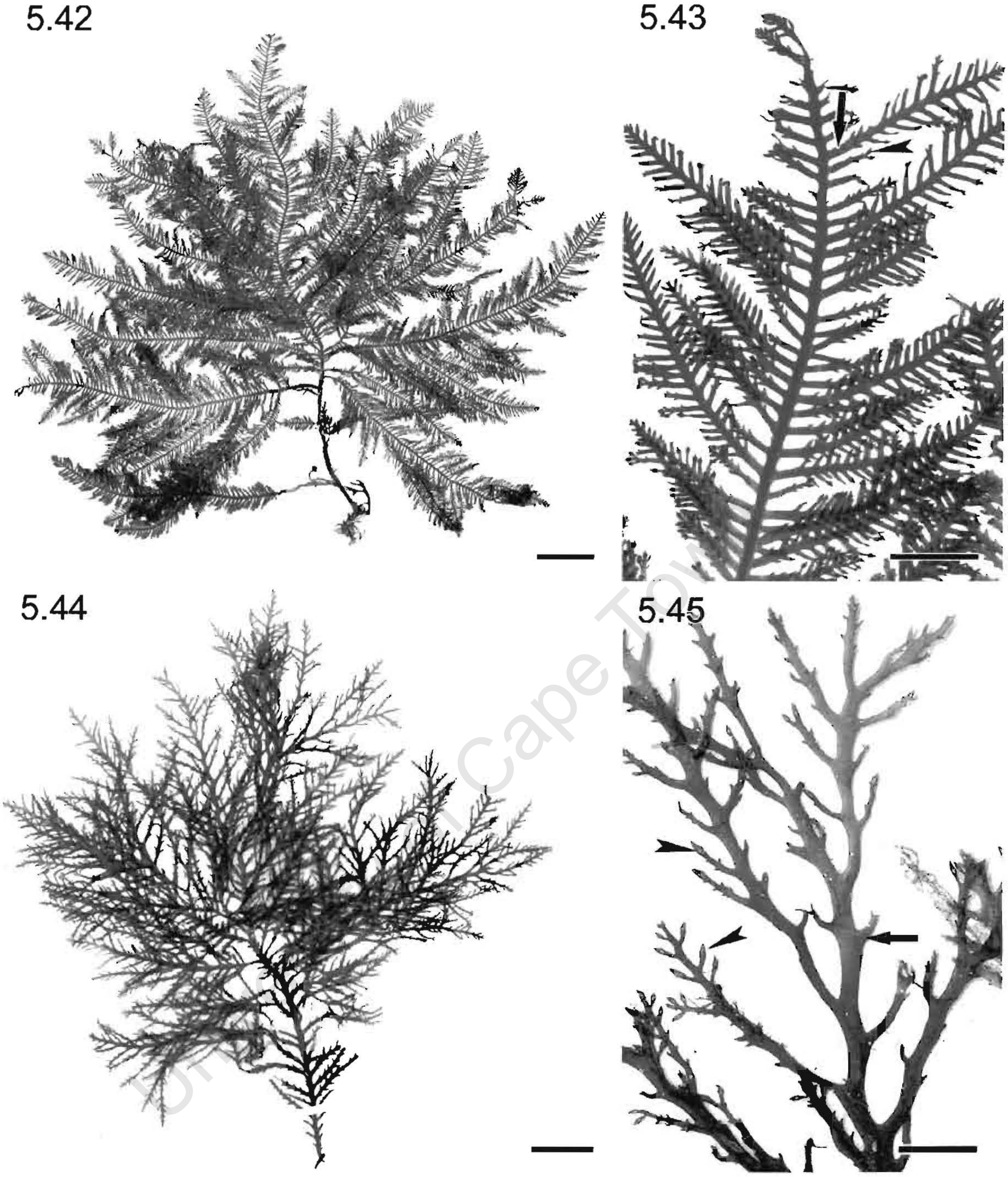


Fig. 5.42-5.43. *Ptilophora scalaramosa*. Fig. 5.42. Habit. Scale = 3 cm. Fig. 5.43. Primary branch bearing evenly spaced second order branches (arrow) on which ramuli commence at a very uniform distance from the junction with the primary branch, conferring a ladder-like appearance to the frond from which the species got its name. Cystocarps borne subterminally on ultimate pinnae (arrowhead). Scale = 1 cm. Fig. 5.44-5.45. *Ptilophora subcostata*. Fig. 5.44. Habit. Scale = 2 cm. Fig. 5.45. Second order branch (arrow) zigzagging between alternately arranged third order branches, an effect created by the broad triangular bases of the branches which are decurrently united with the bearing branch and have acute axils. Cystocarps (arrowhead) borne subterminally on ultimate pinnae. Scale = 1 cm.

Description

Plants up to 1 m long. Blade compressed to flattened, 0.2-5 mm broad, midribbed only in lower parts. Branching distichous, up to four times pinnate. Ultimate ramuli subulate. Branches variably spaced, ca. 2.5 (-8) mm between primary branches. Branches arising from a triangular base with acute axil, when alternately arranged they may make the bearing branch appear to zigzag slightly between branches. Proximal axis margins bear basal branch stubs. Vegetative structure is four-layered. Cystocarps and tetrasporangia borne terminally or subterminally, soral area may bear constrictions, tetrasporangia cruciate. Cystocarps bilocular, borne subterminally or basally on branchlet. Spermatangia unknown.

Distribution

This species has been reported from southern Japan, along the coast of the Miyazaki Prefecture (formerly Hiuga Province), and central Japan, from the coast of Mie (formerly Ise and Shima Provinces), northern Shizuoka (formerly Izu Province), Kanagawa (formerly Sagami Province) and southern Chiba (formerly Awa Province) Prefectures (Okamura, 1909), and Sikine-zima, Kôzu-sima and Izu-ôsima islands (Akatsuka 1987).

Specimens examined: Ise Prov. (Japan); Yamada; vii 1930; s.n. (S). Awa Prov., Shirahama (Japan); 28 vii 1911; s.n. (LD – Herb. Agardh).

Molecular Analyses

According to the calculated *rbc*L sequence divergences (Table 5.1), *Ptilophora helenae* is distantly related to *P. pectinata* relative to its relatedness to *P. leliaertii*, its sister taxon (Fig. 3.24 in chapter 3). *Ptilophora hildebrandtii* is more closely related to *P. scalaramosa* than it is related to other South African *Ptilophora* species. *Ptilophora hildebrandtii* and *P. scalaramosa* are more closely related than *P. pectinata* and *P. helenae*, a result congruent with the calculated LSU sequence divergences as well.

Table 5.1. *rbc*L and LSU gene sequence divergences between *Ptilophora* species

Pairwise comparison	Sequence divergence
*rbc*L data	
P. pectinata vs. *P. helenae*	5.7 %
P. leliaertii vs. *P. helenae*	0.4 %
P. scalaramosa vs. *P. hildebrandtii*	2.6 %
P. hildebrandtii vs. South African *Ptilophora* species	3.8-5.5 %
LSU data	
P. pectinata vs. *P. helenae*	0.77 %
P. scalaramosa vs. *P. hildebrandtii*	0.36%

DISCUSSION

P. hildebrandtii is very similar morphologically to *Ptilophora scalaramosa* (Table 5.2) despite considerable molecular divergence: long branch-lengths between sequences of these species in LSU and *rbc*L phylogenies (fig. 4.24 and 4.25) indicate two discrete taxa. Maximum number of branch orders attained is consistently different between mature thalli of these species, *P. hildebrandtii* having up to two more orders of branches even in thalli half the size of *P. scalaramosa*. Thalli of *P. hildebrandtii* can look considerably more crowded due to a higher degree of lateral indeterminate branch development and by being proportionally smaller. The mean distance between primary branches is 0.7 mm in *P. hildebrandtii* whereas it is closer to 1 mm in *P. scalaramosa*. Ultimate pinnae in *P. hildebrandtii* are frequently as little as half the width of ultimate pinnae in *P. scalaramosa*, being on average around 0.1 mm wide.

Ptilophora helenae and *P. rumpii* are often collected together and despite having pectinate axes of procurrent rostrate determinate branches are clearly distinguishable by the lack of a midrib in *P. helenae* and the broadly alate midrib in *P. rumpii*.

Ptilophora rumpii and *P. pinnatifida* have been found to be closely related molecularly (Fig. 4.24 and 4.25). Despite fundamental differences in pinnule characteristics and, hence, branch development (Table 5.2) it is possible that *P. rumpii* represents an aged form of *P. pinnatifida*. However, *P. rumpii* has yet to be collected growing attached in its true environment and the drift location from where it has been collected (Richards Bay, South African east coast) is about 250 km north of the northern limit of *P. pinnatifida*'s through a seaway across West Antarctica, during periods of minimal glaciation (during Oligocene and Miocene), followed by distribution by high latitude ocean currents to South Africa. The second pathway involves dispersal of algal species from Western Australia to eastern and southern Africa along the North Indian Ocean by prevailing currents during the Miocene to present. Hommersand and Fredericq (2003) provided molecular evidence (inferred phylogenetic hypothesis for six species clusters and pairwise base distances of additional taxa based on analyses of *rbc*L sequences) to substantiate the hypothesised migration of algal species from Australasia to the South African west coast and Namibia via West Antarctica. They also provided molecular data suggesting an algal migration via the North Indian Ocean, but stated that too few examples were analysed to test this hypothesis.

The *rbc*L and LSU phylogenies of chapter three appear to substantiate the hypotheses of Hommersand (1986). *Ptilophora pectinata* occurs along the south-eastern coast of Australia (New South Wales) and in New Zealand. The species was resolved basal to a clade containing the South African *Ptilophora* species in the *rbc*L phylogeny (Fig. 3.24), though there was no bootstrap support for this relationship. Despite the extreme morphological similarity between *P. pectinata* and *P. helenae* from South Africa, the species are distantly related molecularly. The relatively large sequence divergence between *P. pectinata* and *P. helenae* (Table 5.1) suggests a long period of separation between these taxa. Thus, an Australasian origin of *Ptilophora* in South Africa is hypothesised, presumed to have involved dispersal via West Antarctica. In the same *rbc*L phylogeny, *Ptilophora scalaramosa* from the Philippines, Indonesia and Queensland (Australia) was resolved basal to *P. hildebrandtii* from South Africa, to which it bears a striking resemblance. These species are more closely related to one another than *P. hildebrandtii* is related to other South African *Ptilophora* species. Considering the Indian Ocean distributions of *P. scalaramosa* and *P. hildebrandtii* and their molecular relatedness, the occurrence of *P. hildebrandtii* along east Africa is likely to be due to the more recent algal migration from the south-eastern Indian Ocean via the North Indian Ocean sea board. The more recent evolutionary link between these species is reflected in their *rbc*L sequence divergence, which is considerably smaller than that between *P.*

Table 5.2 – Morphological characteristics of *Ptilophora* species.

	Ptilophora leliaertii	***Ptilophora helenae***	***Ptilophora pectinata***	***Ptilophora rumpii***	***Ptilophora pinnatifida***	***Ptilophora coppejansii***
Maximum plant height (cm)	35	40	30	35	35	23
Proximal axis diameter (mm)	2	2	2 (-4)	1.5	2	3 (-6)
Midrib distinct to naked eye in flattened blades	x	x	x	✓	✓	✓
Maximum branch order	6	4	4	4	5	4
Branch width (mm)	0.5-2.5	0.36-1.45	0.2-1.2	0.5-5	0.5-5	0.2-3
Distally*, branches narrower than bearing branch	x	✓	✓	✓	✓	✓
Maximum distance between pinnae (mm)	2.5	2.8	3	2	5	1.3
Distal branches concave/undulate/flattened	concave	flattened	flattened	flattened	flattened	flattened
Branch margins	smooth	smooth	smooth	smooth to eroded	smooth	smooth
Shape of ultimate pinnae	linear with obtuse/acute tip	rostrate to acuminate	rostrate to acuminate	triangular to linear to rostrate, acute to obtuse apex, procurrently curved	triangular to linear to cuneate with truncate apex, procurrently curved	linear with rounded/acute tips
Shape of uninjured indeterminate branch apex	acute to tridentate	acute to tridentate	acute to tridentate	rounded to tridentate	Truncate to tridentate	broadly and bluntly tridentate
Proximal axis margins notched (bearing basal branch stubs)	x	✓	✓	x	x	x
Branching pattern	compound pinnate	pectinate axes, compound pinnate in places	pectinate axes, compound pinnate in places	major branching irregularly, pectinate in places	compound pinnate	pinnate, sparse indeterminate branches

*In broadly alate species, ala can become eroded and reduced to the midrib proximally not giving a true indication of branch width.

Table 5.2 (cont.) – Morphological characteristics of *Ptilophora* species.

	Ptilophora diversifolia	***Ptilophora rhodoptera***	***Ptilophora pterocladioides***	***Ptilophora biserrata***	***Ptilophora mediterranea***	***Ptilophora prolifera***
Maximum plant height (cm)	30	35	25	22	11	45
Proximal axis diameter (mm)	2.5 (-6)	3.5	1.5-2	1.8	2	4 (-7)
Midrib distinct to naked eye in flattened blades	✓	✓	x	x	✓	x
Maximum branch order	4	3	6	3	4	4
Branch width (mm)	2-5	3-10	0.5-2	1-2.5	1-5	0.5-7
Distally*, branches narrower than bearing branch	x	x	✓	✓	✓	✓
Maximum distance between pinnae (mm)	not pinnate	not pinnate	1-10	2	6	4
Distal branches concave/undulate/flattened	undulate	undulate	flattened	flattened	flattened	concave/flattened
Branch margins	undulate to crenate to eroded	blunt or mucronate serrations	smooth, frequent constrictions	serrate	smooth	smooth
Shape of ultimate pinnae	not pinnate	not pinnate	oblanceolate to linear	linear, obtuse apex	mucronate serrations	simple, curved, rounded apex
Shape of uninjured indeterminate branch apex	rounded	rounded, retuse to emarginate	rounded	acute to rounded	rounded	rounded
Proximal axis margins notched (bearing basal branch stubs)	x	x	x	x	x	x
Branching pattern	irregularly & sparsely branched	irregularly & sparsely branched	irregular, sparse	irregular, sparse	compound pinnate	procurrent pinnate axes, compound pinnate in places

*In broadly alate species, ala can become eroded and reduced to the midrib proximally not giving a true indication of branch width.

Table 5.2 (cont.) – Morphological characteristics of *Ptilophora* species.

	Ptilophora scalaramosa	***Ptilophora hildebrandtii***	***Ptilophora subcostata***	***Ptilophora irregularis***
Maximum plant height (cm)	30	40	60	64
Proximal axis diameter (mm)	2	2	3	4
Midrib distinct to naked eye in flattened blades	x	x	x	x
Maximum branch order	4	6	4	3
Branch width (mm)	0.1-2	0.06-1.5	0.2-5	0.2-4.1
Distally*, branches narrower than bearing branch	✓	✓	✓	✓
Maximum distance between pinnae (mm)	3	3	8	2-63
Distal branches concave/undulate/flattened	flattened	flattened	flattened	flattened
Branch margins	smooth	smooth	smooth	smooth
Shape of ultimate pinnae	apiculate	apiculate	subulate	lanceolate
Shape of uninjured indeterminate branch apex	acute/bi-/tridentate	acute/bi-/tridentate	acute	acute
Proximal axis margins notched (bearing basal branch stubs)	✓	✓	✓	x
Branching pattern	compound pinnate	compound pinnate	compound pinnate	irregular, sparse

*In broadly alate species, ala can become eroded and reduced to the midrib proximally not giving a true indication of branch width.

CHAPTER 6

THE DIVERSITY OF THE SOUTH AFRICAN GELIDIACEAE (RHODOPHYTA)

INTRODUCTION

Kylin (1956), Norris (1987a, 1987b, 1990a, 1990b, 1992b), Norris *et al.* (1987), Papenfuss [1940, 1952, notes (unpublished)] and Stegenga *et al.* (1997) made very valuable contributions to the systematics of the South African Gelidiaceae, Norris (1992b) in particular having compared South African collections to type material. This previous research has greatly improved our understanding of the diversity and South African distribution of this algal family. However, it is believed that there is still much scope for systematic research on the South African Gelidiaceae. Plants belonging to the Gelidiaceae with a small habit (< 5 cm) have often been ignored by field collectors due to the difficulty in their identification. Species in the family exhibit considerable phenotypic plasticity and often lack single discrete defining characters, generally being identified by a combination of characters which are often very limited in collected material and often require microscopic observation to be determined. Collections have often been misidentified as a result, particularly with respect to turf-form species where external morphology is sometimes exceedingly similar across different genera, such as thalli of *Gelidium* Lamouroux and *Pterocladiella* Santelices *et* Hommersand. In such instances, cystocarpic material becomes essential in determining which genus specimens belong to, but gametophytic plants are seldom collected, because vegetative and tetrasporangial plants are more common.

The description of new species in the Gelidiaceae based solely on thallus morphology is problematic since it is difficult to ascertain consistent morphological differences that do not fall into the range of morphological variation of related taxa. Since single diagnostic characters are hard to find in the Gelidiaceae, new species have often been lumped into other existing species concepts, thereby extending their range of morphological variation, for example *Gelidium pusillum* (Stackhouse) Le Jolis and *G. latifolium* (Greville) Bornet *et* Thuret from Europe (Fredriksen *et al.*, 1994). This in turn makes the recognition of new species difficult since existing species may not be clearly defined.

Molecular systematics has become a tremendously useful tool in the taxonomy of the Gelidiaceae since the molecular divergence between related taxa is very low relative to the environmentally determined morphological variability seen in members of this group. Intraspecific sequence divergence has been found to be usually below 1% in most genera in the Gelidiaceae (Freshwater and Rueness 1994, Shimada *et al.* 1999, Chapters 1 and 3). Sequence analysis has consequently improved our understanding of morphological variability within taxa and helped in identifying specimens that are less than optimal (the specimen is juvenile, grazed, degraded or vegetative), provided numerous closely related taxa are included in the sequence analysis. Through the sister relationships formed by a sequence when included in a phylogeny, a positive genus identification can usually be made even in the absence of cystocarpic material, since current research is starting to show that genera in the Gelidiaceae form clades (chapters 2 and 6). The sequence divergence between specimens can then be used as an indication of whether they are the same or a different species. Molecular sequence analysis becomes an increasingly more powerful identification tool as the number of related sequences used in an analysis are increased, provided that the morphological work is done comprehensively and thoroughly.

Molecular systematics were not used in the previous research on the South African Gelidiaceae. This chapter takes a combined molecular and morphological approach to further resolving the diversity of South African Gelidiaceae, by finding congruence between generated molecular phylogenies and morphological data gathered from new and old collections, as well as type material. Two new species, *Gelidium declerckianum* and *G. profundum*, and one new record for South Africa, *Pterocladiella bartlettii* Taylor, are described here. The legitimacy of *rbc*L sequences derived from specimens of *G. reptans* (Suhr) Kylin and *P. caespitosa* (Kylin) Santelices, is determined by comparisons of the specimens with type material. Three additional species are also described here that require further taxonomic research, *Gelidium* sp., *P. 'caerulescens'* and *Pterocladiella* sp. These represent possibly a new genus, a new record for South Africa and a new species respectively. South African collections of *Gelidium arenarium* Kylin, *G. crinale* (Turner)

Gaillon, *G. isabelae* Taylor and *G. minusculum* (Weber-van Bosse) Norris are also discussed.

MATERIALS AND METHODS

Specimens were collected from the intertidal and from deep subtidal reefs (using SCUBA) at various locations along the South African coastlines from 1999 to 2002. Specimens were pressed, dried with silica gel or wet preserved in 5% Formalin in seawater. Specimens, including type material, were loaned from various national and international herbaria.

Molecular Analyses

DNA was extracted from 19 silica-gel-dried or pressed specimens. For the protocol used for total genomic DNA extraction, *rbc*L gene amplification and sequencing, sequence alignment and the determination of data characteristics, refer to the materials and methods section of chapter 1. These sequences were included in an *rbc*L data file containing 37 additional sequences (of which 35 had been previously published). Twenty seven of the 56 sequences were from South African specimens, including 15 of the 21 Gelidiaceae species reported to occur in South Africa prior to this thesis. Sequences representative of all *rbc*L clades resolved to date in various publications (Freshwater *et al.* 1995, Freshwater and Rueness 1994, Shimada *et al.* 1999, Shimada and Masuda 2000) were included in the data file in order to have as optimal a representation as possible of the genetic diversity within the Gelidiaceae, but limited to a total of 56 sequences for the analyses to be practical with respect to the time it would take to run them. Refer to the CD-ROM attached for an *rbc*L gene data file including all the sequences analysed in this thesis.

Distance, maximum parsimony and maximum likelihood analyses were performed on an *rbc*L data file of 56 specimens using PAUP (v. 4.0b10, Swofford 2001). Due to missing data at the 5' ends of *rbc*L sequences, the first 67 sites of the 1467 bp *rbc*L gene were excluded from the analysis such that at least 50% of the sequences had complete data at the 5' end. Distance trees were generated using neighbour-joining tree building with Tamura-Nei distances. The Tamura-Nei correction was used since the data set had an unequal frequency of bases and a pyrimidine-pyrimidine transition bias. Distance bootstrap analyses consisted of 2000 replicates of neighbour joining tree building with Tamura Nei distances. Maximum parsimony analyses consisted of a heuristic search performing 1000 random sequence additions (holding 50 trees at each step during stepwise addition) using the tree-bisection-reconnection (TBR) branch-swapping algorithm with MULTREES and STEEPEST DESCENT options in effect. A second search starting with the trees found in the initial search was performed using MULTREES, STEEPEST DESCENT and TBR. Maximum parsimony bootstrap analyses consisted of 1000 replications of heuristic searches with 10 random sequence additions, MULTREES, STEEPEST DESCENT, and TBR. Optimality criteria for likelihood analyses were determined with Modeltest v. 3.06 (Posada & Crandal 1998). A maximum likelihood analysis consisted of five random sequence additions with TBR branch swapping and MULTREES. A Bayesian analysis was conducted with MrBayes (vs. 3.0b4; Huelsenbeck and Ronquist, 2001) using six simultaneous Markov Chains and a codon position model incorporating model priors (GTR + I + G) determined by Modeltest. The analysis was run for 1,000,000 cycles, sampling every 100 generations. MrBayes was used to find the best tree from the 10,000 trees saved during the analysis, excluding the first 1,000 which preceded the stabilisation of the likelihood value (the burn-in). Bayesian posterior probabilities were determined by a 50% majority rule consensus. Quartet-puzzling analyses of 1000 puzzling replications were performed using the same model parameters as in likelihood searches.

Morphological Observations

Observations were made using a Wild stereo dissecting photomicroscope and photos were taken with an Olympus Camedia digital camera. Transverse sections were cut from specimens by hand, stained with aniline blue stain and preserved in a 50% Karo^{TM}

solution. Photos were taken with an Olympus DP50 digital camera mounted on a Leitz Diaplan compound microscope.

Morphometric and Statistical Analyses

Sixty branch angles per specimen were measured in four specimens of *G. pteridifolium* and four of *G. profundum* using digital image analysis software (UTHSCSA Image Tool). A Mann-Whitney U Test for independent variables was performed on the pooled branch-angle data (n=240) for both species to test for a significant difference between samples. This nonparametric test was used because the data were not normally distributed for one of the variables and could not be normalized with various types of data transformations.

RESULTS AND DISCUSSION

*rbc*L Analyses

The analysed *rbc*L data set included 1400 nucleotide sites. Five hundred and forty five sites (38.9%) were variable of which 467 (33.4%) were parsimony-informative. The data set had an unequal frequency of bases (A=30.9%; C=16.9%; G=21.3%; T=31%) and a transition:transversion ratio of 3.43 with a 61.5% bias towards pyrimidine-pyrimidine transitions. The topologies derived from all four tree-building methods were very similar. A maximum parsimony analysis of these data produced nine trees with a length of 2056 steps and consistency index of 0.4 (Fig. 6.1). A Maximum Likelihood analysis resolved two trees. The Shimodaira-Hasegawa test did not find a significant difference between the log likelihood values of the two trees (p=0.499).

The topology of the distance, Bayesian and maximum likelihood trees differed from the maximum parsimony tree in the relationships between species within the '*Gelidium coulteri* clade', the 'Indo-Pacific/Caribbean Clade', the clade containing *Pterocladiella caerulescens* (Kützing) Santelices *et* Hommersand and *P. beachiae* Freshwater, and in the placement of the unnamed species "*Gelidium sp.*" from Sodwana (South Africa) in

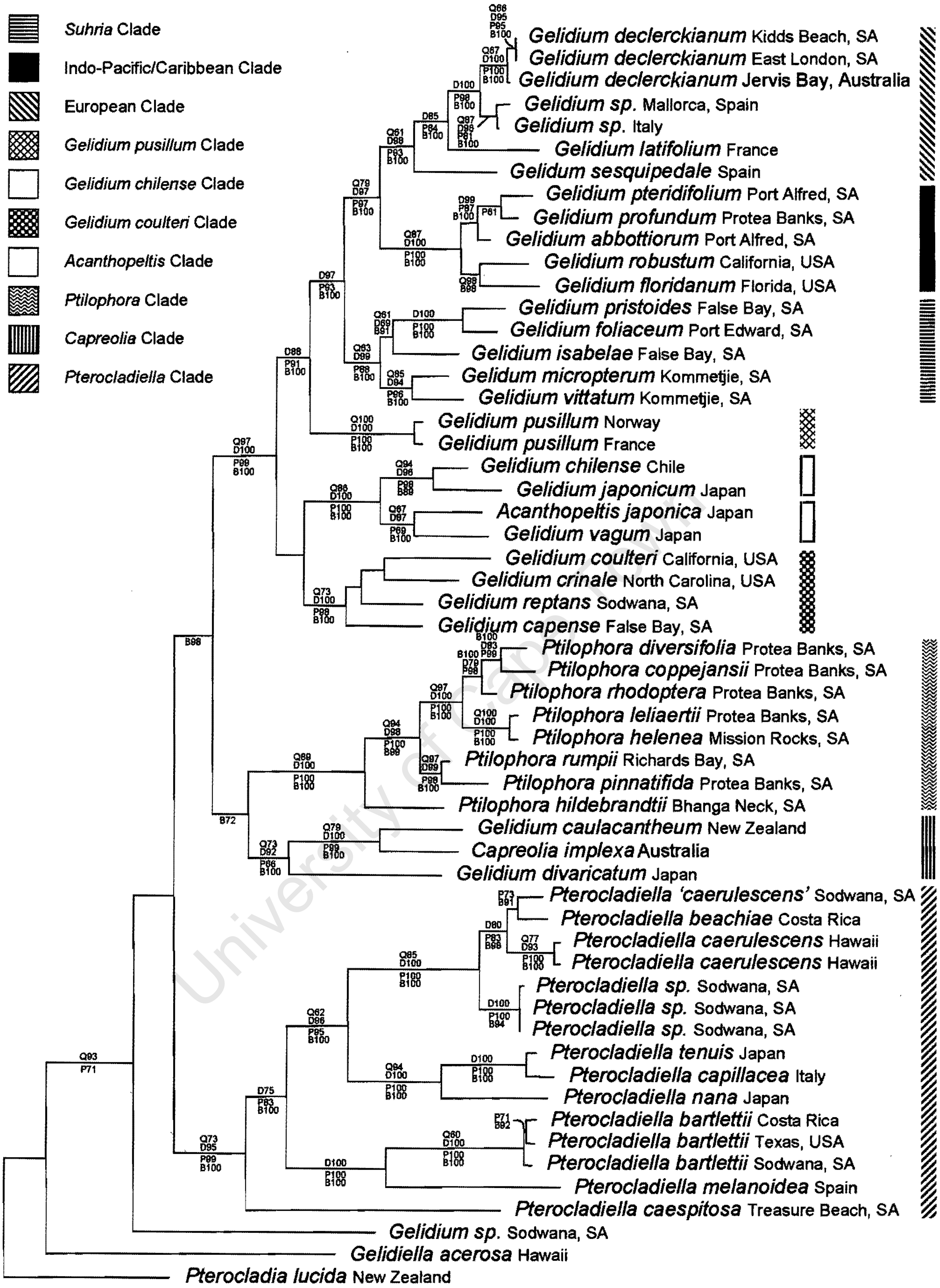


Fig. 6.1. One of nine most parsimonious trees of length 2056 (CI = 0.4). Estimates of branch support included when ≥ 50%. Parsimony bootstrap (P), distance bootstrap (D), Bayesian reliability (B), quartet puzzling reliability (Q). Scale = 10 changes.

the tree. These differences, except those within the '*Gelidium coulteri* clade', will be discussed in more detail in the following sections. The differences within the '*Gelidium coulteri* clade' are irrelevant to the aims of this chapter.

With respect to sequences derived from South African taxa, all analyses resolved *G. capense* (Gmelin) Silva and *Gelidium reptans* in the '*Gelidium coulteri* clade', *Gelidium pteridifolium* Norris, Hommersand *et* Fredericq and *Gelidium abbottiorum* Norris in the 'Indo-Pacific/Caribbean clade'; *Gelidium pristoides* (Turner) Kützing, *Gelidium foliaceum* (Okamura) Tronchin, *Gelidium vittatum* (Linnaeus) Kützing, *Gelidium isabelae* Taylor and *Gelidium micropterum* Kützing in the '*Suhria* clade'; *Pterocladiella caespitosa* (Kylin) Santelices in the '*Pterocladiella* clade' and species of *Ptilophora* together in a monophyletic clade. Two new species, *Gelidium declerckianum* and *Gelidium profundum*, was resolved in the 'European clade' and 'Indo-Pacific/Caribbean clade' respectively. A new record, *P. bartlettii* Taylor was resolved in the *Pterocladiella* clade. Five other collections possibly representing three new taxa were included in the analyses. Two of these, *Pterocladiella 'caerulescens'* and *Pterocladiella* sp., were resolved in the '*Pterocladiella* clade'. The third taxon, *Gelidium* sp., was resolved as a basal lineage.

Morphology and Taxonomy

Gelidium foliaceum, *G. pristoides G. vittatum* and the species of *Ptilophora* were taxonomically treated in previous chapters and will not be discussed further. *Gelidium micropterum* is believed to be a discrete, well-described species and will also not be discussed here.

Gelidium declerckianum Tronchin *nom. prov.*

Fig. 6.2-6.9

Description: Uprights (2-) 3.5-10 (-15) mm long. Prostrate stolons terete about 125 µm in diameter. Uprights cylindrical to subcylindrical becoming flattened (50-100 (-140) µm thick), broadening distally (200-450 (-600) µm wide). Apices blunt, bi- or tripartite. Irregularly and distichously branched. Surface proliferations common. In the flattened distal parts up to five orders of branches produced; branches crisped, irregularly curled and twisted. Lacerations occur apically and on distal margins. In transverse section, outer cortical cells quadrangular, up to 8 µm in length, as narrow as 5 µm in width, with a length/width ratio of 1-1.5. Inner cortical cells are globose with angular protoplasts, 6-10 µm (mostly ca. 8 µm) in diameter. Rhizines mostly <3 µm in diameter, occur between inner cortical cells and scattered throughout the medulla. Medullary filaments ovoid, up to 17µm in diameter. Spermatangial sori subterminal, on both blade surfaces, surrounded by narrow sterile margin. Tetrasporangial sori subterminal, initially acropetally developed. Tetrasporangia cruciately divided, 22-34 µm long and 22-30 µm wide in surface view, with length/width ratio of 1-1.5. Cystocarps unkown.

Etymology: This species is named after the phycologist Dr. Olivier De Clerck in recognition of his passion and enthusiasm for algal taxonomy and his wonderful ability to communicate it.

Holotype: Plantation Point, Jervis Bay, New South Wales, Australia; A.J.K. Millar; 30 vii 2002; NSW-9 (NSW) - pressed, formalinised, and silica-gel-dried.

Ecology and Distribution: Plants have only been found growing in the intertidal on molluscs and exposed rocks subject to periods of desiccation during low tides. South African plants have only been found at Nahoon Point (East London) and Kidds Beach, two localities about 50km apart along the coast of the Eastern Cape Province of South Africa. Australian plants have only been recorded from Plantation Point, Jervis Bay, New South Wales.

Specimens examined: Kidds Beach, Eastern Cape Province, South Africa; E.M. Tronchin; 6 v 2001; 110878 (BOL), 510 (BOL) wet preserved. Nahoon Point, Eastern Cape Province, South Africa; E.M. Tronchin; 7 v 2001; 99067 (BOL), 509 (BOL) wet preserved. Plantation Point, Jervis Bay, New South Wales, Austrialia; NSW-9.

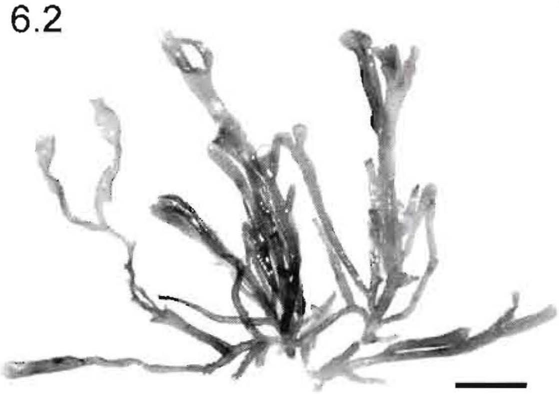
Plants are dark purple in colour, occurring in dense tufts of uprights (2-) 3.5-10 (-15) mm in height united by terete prostrate stoloniferous branches about 125 µm in diameter (Fig. 6.2). Plants may either occur in small patches on mollusc shells and rock surfaces or may form a more expansive turf. Rhizoidal branches issue irregularly along prostrate stolons and are cylindrical, either tapering to an abrupt point or producing at their ends brush-like attachment haptera (Fig. 6.3), composed of thick-walled, aseptate cells, when they contact the substratum. Uprights are cylindrical to subcylindrical basally (50-100 µm in diameter) becoming flattened [50-100 (-140) µm thick], broadening distally [200-450 (-

600) μm wide] and terminating in blunt apices that may be bi- or tripartite (Fig. 6.2), sometimes pseudodichotomous and often lacerated. Lacerations may occur terminally (Fig. 6.4), on either side of the apical cell (Fig. 6.5), or marginally, running longitudinally down the upright. Surface proliferations are common and range from small papillae (Fig. 6.6) to flattened, adventitious branches. Apical cells are dome-shaped and may be flush with the outer cortical layer of cells or may be slightly protuberant. Branching is irregular and distichous, occurring mostly basally on the uprights and close to the apices. In the flattened, distal reaches of the thallus up to five orders of branches may be produced that are crisped and irregularly curled and twisted (Fig. 6.7). Grazed thalli lack the abovementioned extensively branched and irregularly curled distal regions, but may still bear a few uprights that are slightly twisted and curled distally (Fig. 6.8). These thalli are generally more robust, rigid and prolifically branched proximally. Nonetheless, their uprights are still subcylindrical basally (up to 175 μm thick) becoming quickly flattened distally and producing tripartite apices on occasion.

In transverse section, thalli have an outer cortex of quadrangular cells that are up to 8 μm in length and as narrow as 5 μm in width, with a length/width ratio of 1-1.5 (Fig. 6.9). Inner cortical cells are variably shaped, mostly globose and 8 μm in diameter (ranging from 6-10 μm), with angular protoplasts. Rhizines are numerous, mostly <3 μm in diameter, and occur in clusters between inner cortical cells and scattered throughout the medulla. In branch apices, rhizines are few and instead occur individually and scattered throughout the internal tissue. Medullary filaments are round to ovoid in transverse section, thick-walled and up to 17μm in diameter. Cells in the stoloniferous branches can become somewhat larger: outer cortical cells may be up to 10 μm, inner cortical cells up to 12.5 μm and rhizines up to 5 μm in diameter.

Fertile structures have not been found in South African plants. Spermatangial and tetrasporangial thalli have been found in specimens from Australia (D. W. Freshwater pers. comm., 2003). Rounded to obovate spermatangial sori occur subterminally on erect branches. Sori are found on both blade surfaces and are surrounded by a narrow sterile margin. Outer cortical cells undergo two anticlinal cell divisions giving rise to four

6.2



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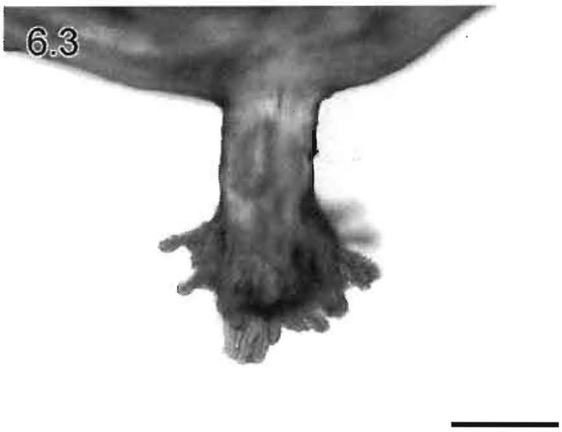

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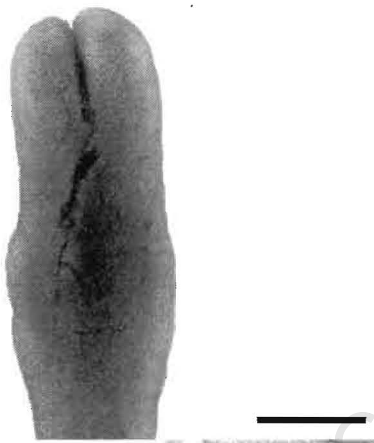

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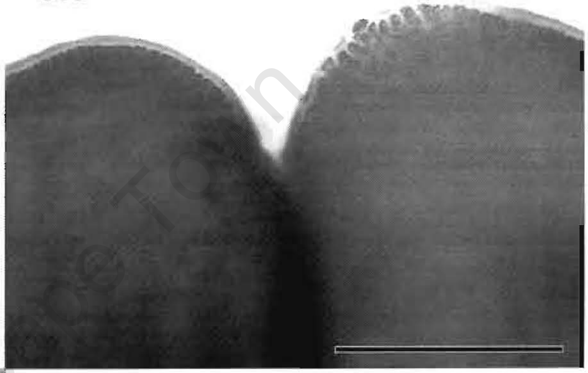

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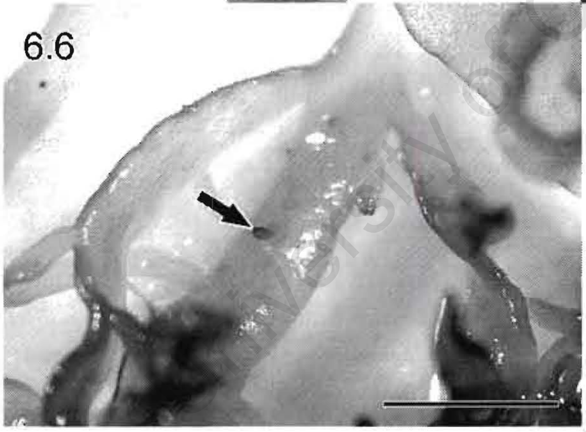

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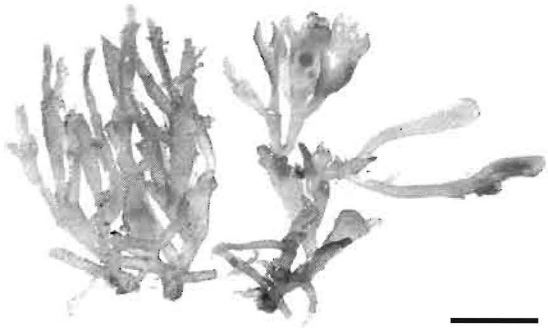

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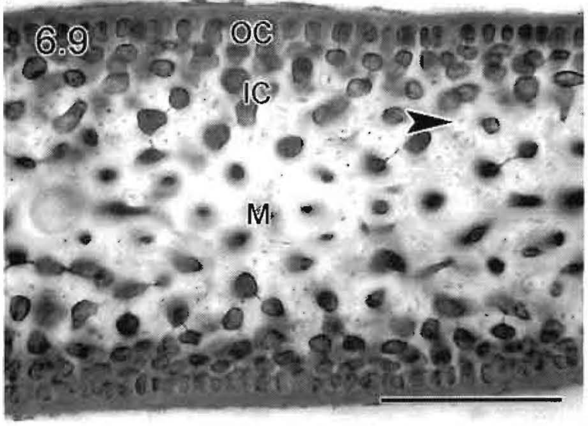

Fig. 6.2-6.9. *Gelidium declerckianum* from South Africa. Fig. 6.2. Habit. Uprights often produce bi- and tripartite flattened apices. Scale = 2 mm. Fig. 6.3. Cylindrical rhizoidal branch (issuing from prostrate stolon) terminating in brush-like hapteron of elongate, aseptate cells. Scale = 100 µm. Fig. 6.4. Distal region of upright developing an apical laceration. Scale = 400 µm. Fig. 6.5. Magnification of the upright in figure 6.4 showing early stages of apical laceration forming two branches. Only one branch has an apical cell. Scale = 100 µm. Fig. 6.6. Simple, papillate proliferation issuing from surface of flattened distal branch (arrow). Scale = 1 mm. Fig. 6.7. Distichously branched upright with irregularly curled and twisted branches. Scale = 2 mm. Fig. 6.8. Grazed habit. The upright system is more robust, rigid and branched. Only a few uprights retain a curled bi- and tri-partite appearance. Scale = 2 mm. Fig. 6.9. Transverse section of flattened upright showing vegetative structure composed of an outer cortex (OC), inner cortex (IC) with interspersed rhizines (arrowhead), and thick-walled, filamentous medulla (M). Scale = 50 µm.

elongated spermatangial initials. Spermatangia are formed by transverse divisions of the spermatangial initials. Tetrasporangial sori occur subterminally on erect branches. Tetrasporangia develop acropetally in young portions of sori, but both mature and developing tetrasporangia are present in older portions of sori. Tetrasporangia are cruciately divided and when viewed from the surface are 22-34 µm long, 22-30 µm wide, with a length/width ratio of 1-1.5. Cystocarpic plants have not been found.

Gelidium declerckianum has characteristics in common with numerous descriptions of *Gelidium pusillum* and its varieties from the world over, but none have exactly the same complement of thallus proportions and cell dimensions. No turf-forming species of the Gelidiaceae has been described which develops uprights with irregularly curled and twisted distal ends, such as are produced by *G. declerckianum*. It must be noted, however, that uprights of *Gelidium reptans* may become twisted and Womersley and Guiry (1994) describes the uprights of *G. pusillum* from southern Australia as having slightly undulate distal margins.

When grazed, this species most closely resembles *Gelidium arenarium* described from Isipingo, KwaZulu Natal Province (KZN), South Africa. Compared to the lectotype of *G. arenarium* (Isipingo, T. A. Stephenson, 13 viii 1935, LD, s.n.) and the descriptions of this species (Kylin 1938, Norris 1992b), it differs primarily in size: axes being consistently thicker and cells consistently smaller, particularly in the outer cortex where the range in cell size (5-9 µm) overlaps that of *G. arenarium* (8-12 µm) by only 2 µm. *Gelidium arenarium* also has mostly cylindrical axes, and it lacks the characteristic curled and twisted distal parts, though figure 2 in Norris (1992b) appears to illustrate two slightly curled apices.

Some uprights of *G. declerckianum* appear to experience a sudden onset of apical proliferation (Fig. 6.7) since they are unbranched or scarcely branched proximally. It is likely that this is promoted by the longitudinal laceration of the flattened apices. Of the two branches resulting from a laceration, only one bears an apical cell early on (Fig. 6.5), the second branch developing an apical cell later and resuming apical growth.

The South African samples of *G. declerckianum* from Kidd's Beach and Nahoon Point have identical *rbc*L sequences which differ from the Jervis Bay, New South Wales sample at only four-nucleotide sites (<0.29%). *Gelidium declerckianum* was strongly supported in all analyses as the sister taxon to an unnamed *Gelidium* species from the Mediterranean. Both these species are resolved within the 'European-Clade' (Fig. 6.1). This is the first species with a distribution outside of the Northeast Atlantic to be resolved in this clade. *Gelidium declerkianum* is only distantly related to other Indo-Pacific Gelidiales species.

***Pterocladiella bartlettii* Taylor, 1943, p. 156; plate 4, fig. 2; 1960, p. 359, pl. 46, fig. 2**
Fig. 6.10-6.11

Type: Saint Lous du Sud, Haiti

Description: Plants up to 13 mm long. Uprights cylindrical to compressed, 0.1-0.25 mm wide, 135-160 µm thick. Prostrate axes cylindrical to compressed giving rise to downwardly-directed cylindrical branches terminating in discoid holdfasts. Rhizines numerous within the medulla. Cystocarps intercalary to subterminal on ultimate branches. Mature cystocarps unilocular, oval to slightly triangular in transverse section, carposporangia radiating from central axis of tissue connected to one of the cystocarp walls and protruding into the cavity.

Ecology and South African distribution: Shallow upper intertidal rock pools, known only from Jesser Point, Sodwana.

Specimen examined: Jesser Point, Sodwana, KwaZulu-Natal Province, South Africa; Freshwater; 11 ii 2001; P22 (BOL) – wet preserved.

Distance, maximum parsimony, maximum likelihood and Bayesian analyses resolved the *rbc*L sequence of a specimen collected from South Africa together with sequences generated from specimens of *Pterocladiella bartlettii* from Costa Rica and Texas in a monophyletic clade (Fig. 6.1) with strong support (bootstrap proportion [BP] = parsimony [P] 100%, distance [D] 100%, Bayesian posterior probability [B] 100%, quartet puzzling reliability [Q] 60%). The *rbc*L data file included sequences from eight of the eleven currently recognised species of *Pterocladiella* for which the sequence divergence between species ranged from 1.9-12.5%. *Pterocladiella bartlettii* from Texas was 0.44% sequence divergent from *P. bartlettii* from Costa Rica, these populations in turn differing from the South African population by 0.5% sequence divergence despite the considerable geographic distance between populations. Hence the *rbc*L data strongly suggests that the South African, Texan and Costa Rican populations are conspecific.

The habit of plants from the South African collection (Fig. 6.10) differs from some published descriptions of *P. bartlettii* (Edwards 1977, Santelices 1998, Taylor 1943, 1971, Thomas & Freshwater 2001) in that uprights are cylindrical to compressed and irregularly and sparsely branched, whereas *P. bartlettii* uprights are compressed to flattened producing a bilateral series of branchlets along the margins of axes, a diagnostic character for this species. However, Thomas and Freshwater (2001, pg. 346, fig. 13) illustrated a small (<2.5 cm) *P. bartlettii* specimen very similar in habit to the South African collection, stating that some of their Costa Rican collections had specimens with terete branches. The original description of *P. bartlettii* by Taylor (1943) states that uprights are at first erect, linear-lanceolate, simple up to 1 cm in length, soon developing into the adult ramification where axes are up to 8 cm tall bearing alternate bilaterally numerous subsidiary axes which become erect and more or less entangled below, free above. These main and subsidiary axes become closely beset with generally divaricate, occasionally ascending, branchlets in bilateral series along the margins. The South African plants would seem to represent the beginning stage of the adult ramification of *P.*

bartlettii described above, both in the size of the thalli and their branching pattern. The South African collection of this species is unfortunately very limited, being only a small tuft of uprights. Thus, the range in morphology in the South African plants is unknown. The South African plants fit within the thallus proportions previously published for P. *bartlettii*, particularly with respect to the branch width and thickness, being 0.5 mm and 125-170 μm respectively according to Taylor (1943), and 0.6 mm and 150 μm respectively according to Santelices (1998). Outer cortical cells are (8-) 10 (-13) μm long in surface view which agrees with the average length of 11.3 μm published by Felicini and Perrone (1994). Rhizines are also restricted to the medulla, a characteristic of *P. bartlettii* (Thomas and Freshwater 2001, Taylor 1943)(Fig. 6.11). The position of cystocarps in thalli of *P. bartlettii* is not well known. Taylor (1943) merely reports their occurrence in generally flat, ultimate branchlets. Cystocarps in the South African specimens have an intercalary to subterminal position on ultimate branches. Cystocarps are known to occur subterminally in other *Pterocladiella* species. The unilocular cystocarp structure is typical of *Pterocladiella* (Fig. 6.12).

There is sufficient morphological and molecular data to recognise this South African collection as representative of *P. bartlettii*. It is the first report of this species from South African shores.

Gelidium reptans, G. isabelae and *G. minusculum*

Gelidium reptans is a sand-binding turf alga characterized by short (0.5-2 cm tall), simple and ligulate uprights (up to 1 mm wide) with rounded apices that become irregularly to pinnately branched. It occurs in upper intertidal, shallow rock pools and on large flat rocks exposed to long periods of desiccation during spring low tides. It may be found in the shallow subtidal as well, in sandy patches and gullies. It is easily distinguishable by the simple short uprights extending above the sandy substratum that conceals its prostrate system. A collection was made from high intertidal exposed rocks at Jesser Pt. (Sodwana, South Africa) where this species forms a dense mat of short fronds easily spotted by the slightly orange hue of the mat. Specimens from this collection (Fig. 6.13) were checked

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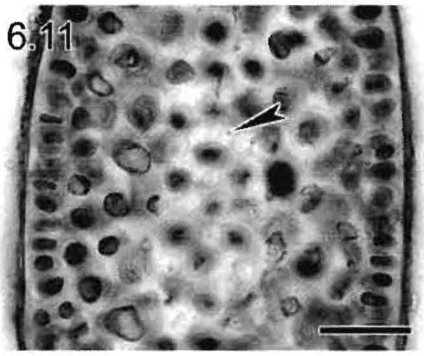

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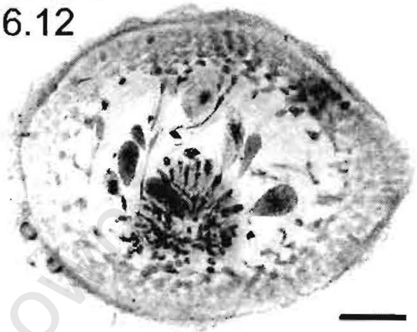

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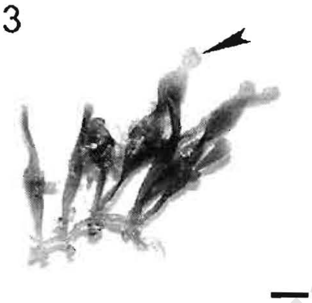

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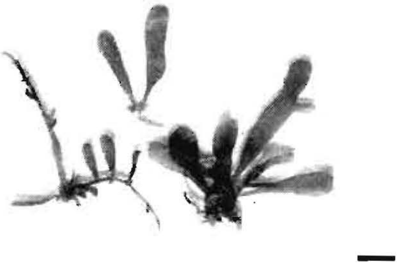

Fig. 6.10-6.12. *Pterocladiella bartlettii*. Fig. 6.10. Habit of specimen from Sodwana bearing intercalary cystocarps (arrowhead) on ultimate branches. Scale = 2 mm. Fig. 6.11. Transverse section of compressed primary branch. Rhizines are restricted to the medulla (arrowhead). Scale = 30 µm. Fig. 6.12. Transverse section of unilocular cystocarp. One side of the placental core of nutritive cells and gonimoblast filaments is attached to the floor of the cystocarp, producing carposporangia from the remaining sides. Scale = 50 µm. Fig. 6.13-6.15 *Gelidium reptans*. Fig. 6.13. Habit of specimen from upper intertidal rocks at Sodwana, bearing terminal tetrasporangial sori (arrowhead). Scale = 1 mm. Fig. 6.14. Habit of isotype specimen at LD. Scale = 1 mm. Fig. 6.15. Habit of pinnately branched specimen from Rocky Bay, KwaZulu-Natal. Scale = 3 mm. Fig. 6.16. *Gelidium isabelae*. Habit of specimen from Skoenmakerskop, Port Elizabeth. Scale = 1 mm.

against the isotype specimen of *G. reptans* (Fig. 14)(*Phyllophora reptans* Suhr, Herb. Agardh, 33266, LD). Despite the degraded state of the type specimen, its overall shape, branching pattern, holdfast morphology, and blade height, width and thickness agreed very well with the habit of the Sodwana collection and they are believed to be conspecific. The *rbc*L sequence generated from a specimen of the Sodwana collection was resolved in the '*Gelidium coulteri* clade' (Fig. 6.1)

The *Gelidium reptans* collections from South Africa are thought possibly to represent two forms: an upper intertidal form that bears mostly short (ca. 6 mm) blades off a stoloniferous holdfast (Fig. 6.13, 6.14); and a lower intertidal to shallow subtidal form (in rock pools and on shallow subtidal sandy patches) that is taller (up to 2 cm), broader (ca. 0.9 mm as opposed to 0.6 mm broad) and pinnately branched (Fig. 6.15). Both forms are illustrated under the name *G. reptans* in Norris (1992b: pg. 20, fig. 9a-f). The first form can be found on exposed upper intertidal rocks or on limpet and barnacle shells. The blades are usually twisted and sometimes slightly spatulate in which case the terminal lobe bears an ill-defined tetrasporangial sorus. This form is distributed from Isipingo to Sodwana and agrees morphologically with the type specimen of this species. The second form looks like the adult plant of the first form, due to its added height and breadth and the occurrence of pinnate branching. However, even small (<1 cm tall) erect blades of this form are broader (up to 0.5 mm) than those of the first form, and are pinnately branched. The tetrasporangial sori are usually well defined and surrounded by a distinct sterile margin in surface view. This form has only been collected along central KZN from Mvoti in the north to Rocky Bay (Park Rynie) in the south. Molecular sequencing of the second form could not be carried out in this study due to time constraints.

In figure 1.1 (chapter 1) *Gelidium reptans* was resolved in the '*Suhria* clade' of the *rbc*L phylogeny. This *rbc*L sequence was generated by D.W. Freshwater from a sample from False Bay, South Africa collected by M. Hommersand. The sample is now believed to represent *G. isabelae* (Millar and Freshwater, 2004), a species originally described from Isla Isabela in Ecuador (Taylor, 1945) and reported from the coast of New South Wales, Rottnest Island, Lord Howe Island (Australia), New Caledonia (D.W. Freshwater, 2003;

pers. comm.) and the Pacific coast of Columbia (Schnetter and Bula Meyer, 1982). *Gelidium isabelae* was resolved in the '*Suhria* clade' of the *rbc*L phylogeny of this study as well (Fig. 6.1). The species is also known from Skoenmakerskop, Port Elizabeth, South Africa (Fig. 6.16).

Gelidium minusculum from South Africa is only known from two pressed specimens of cultured material (Nat 2440, NU) from Norris's culture collection, which are characterised and illustrated in Norris (1992b). The habit of cultured plants can be considerably different from that of plants *in situ*. A cultured specimen of *G. foliaceum* from NU has a terete, irregularly branched, filamentous habit which is completely unlike the crispate, ligulate and marginally lobed blades of *in situ* material of this species. Field-collected specimens of *G. minusculum* were not found at BOL, GRA, NU or Norris's wet collection housed at the Department of Animal, Plant and Environmental Sciences of the University of the Witwatersrand. Hence, the identity of NAT 2440 and the report of *G. minusculum* from South African shores could not be confirmed and remains questionable. The *rbc*L sequence of *G. isabelae* from South Africa is very similar to the *rbc*L sequence of a specimen of South African *G. minusculum* from the culture collection of Prof. M. Guiry and Dr. J. Rico, which was obtained from Norris's cultured material (fide M. Guiry). Thus, it is possible that *G. minusculum sensu* Norris (1992b) is in fact *G. isabelae*.

Specimens examined: Gelidium isabelae: Skoenmakerskop, Port Elizabeth; Beckley; 7 ii 1981; 5221 (BOL). *Gelidium minusculum:* Culture - Rocky Bay, Natal, South Africa; Pienaar; 28 viii 1984; NAT 2440 (NU). *Gelidium reptans:* Isotype - Cap. b. Spei.; Suhr, 33266 (Herb. Aghard, LD). Durban (Isipingo); Stephenson; s.n. (loan no. 86/76-5818, LD). Bhanga Neck (Boteler Pt.); Tronchin; 13 viii 1999; KZN 121 (BOL / GENT). Sodwana, Jesser Point, South Africa; Tronchin; 10 ii 2001; P20 (BOL) – wet preserved. Rocky Bay, Natal; Aken and Norris; 9 ix 1983; NAT 1423 (NU).

Gelidium crinale and *G. arenarium*

A number of specimens of *Gelidium crinale* have been deposited at BOL, NU and GRA by various collectors. The specimens were collected from the Eastern Cape coast (Cape

Morgan in particular) and False Bay. Specimens are mostly terete, sometimes flattened distally and up to 6 cm in length (Fig. 6.17). Although small specimens (~2 cm tall) can be confused with adult plants of *G. arenarium*, branching in *G. crinale* is irregular, often restricted to the apical half of the erect axes and appearing as dichotomies rather than as pinnules, and axes are broad at the origin of branches; in agreement with the description of this species in Santelices (1977). *Gelidium arenarium* branches sparsely and irregularly throughout instead, axes often being compressed basally becoming terete distally where branches are oppositely arranged and end in tripartite tips; a distinguishing feature of this species (Fig. 6.18). The identity of specimens of *G. arenarium* from South Africa was checked by comparison with lectotype material at LD (Fig. 6.19). This species has been repeatedly collected from Arniston, Western Cape Province.

A specimen (no. 2114a) housed in the Pocock collection at GRA was identified as *Pterocladia heteroplatos* (Børgesen) Umameshwara Rao *et* Kaliaperumal by Norris. The specimen was collected at St. Lucia Rocks in KZN, and would be the first record for this species in South Africa. The specimen is tetrasporangial and without cystocarpic material its identification as a species of *Pterocladia* is debatable. The specimen exhibits pseudodichotomous branching as in *Gelidium crinale* though *Pterocladia heteroplatos* is reported to have distichous, alternate to opposite branching (Umameshwara Rao and Kaliaperumal, 1980). Axes are also never more than 400 μm in width whereas axes of *P. heteroplatos* range from 250 μm to 800 μm in width. In a letter accompanying the specimen, written by Norris presumably to the curator of GRA, suggests that the identity of the specimen had been debated for some time: "...I changed my mind on its identity – I think this must be the last word!" Nonetheless, Norris's final word still lacked conviction. This specimen is thought to represent *Gelidium crinale* (based in the style of branching) rather than *Pterocladia heteroplatos* and will be treated as such in this study.

Specimens examined: Gelidium arenarium: Lectotype - Durban, Isipingo; Stephenson; 13 vii 1935; s.n. (loan no. 2/0170441, LD). Arniston; Pocock; 21 ii 1939; 606 (GRA). Tergniet; Isaac; 14 x 54; I328 (BOL). Muizenberg, False Bay; Papenfuss; 20 i 1939; 187 (NU). *Gelidium crinale:* Cape Morgan; Flanagan; 74 (36576, BOL). St. Lucia Rocks; Pocock; 21 vii 1938; 2114a (GRA).

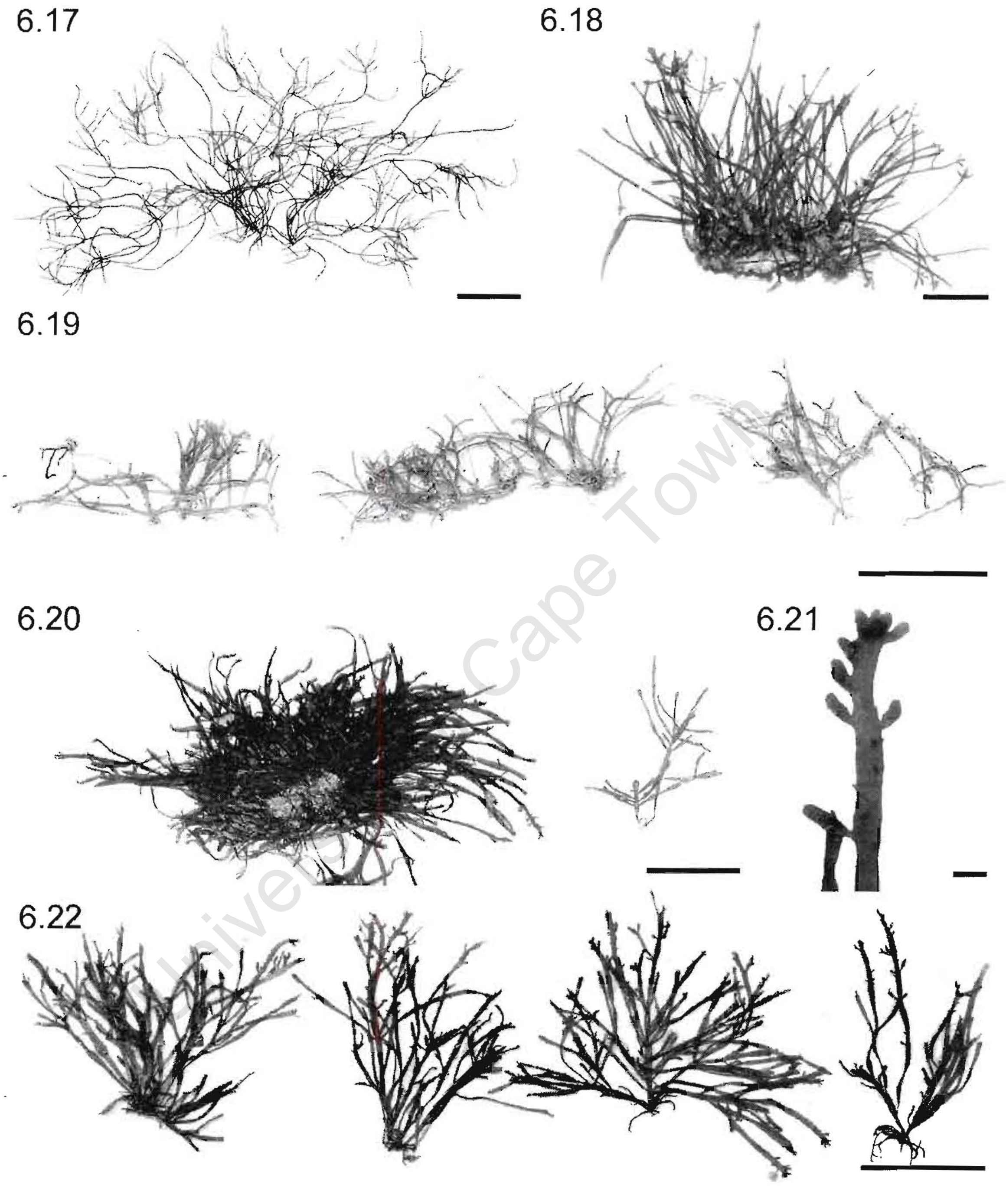

Fig. 6.17. *Gelidium crinale*. Habit of specimen from Cape Morgan. Scale = 2 cm. Fig. 6.18-6.19. *Gelidium arenarium*. Fig. 6.18. Habit of adult plant of from Arniston. Scale = 1 cm. Fig. 6.19. Habit of lectotype at LD. Scale = 1 cm. Fig. 6.20-6.22. *Pterocladiella caespitosa*. Fig. 6.20. Caespitose habit of specimen from Isipingo with pinnately branched upright extracted from tuft, appearing alongside it. Scale = 1 cm. Fig. 6.21. Distal axis bearing short branchlets in a bilateral series near the apex. Scale = 400 µm. Fig. 6.22. Habit of type material at LD. Scale = 1 cm.

Pterocladiella caespitosa

Pterocladiella caespitosa is a small (up to 3 cm), tufted alga occurring in the lower intertidal. It is characterized by the occurrence of short bladelets in a bilateral series towards the apices of axes or distal branches (Fig. 6.21). A specimen of this species collected from Treasure Beach near the type locality, Isipingo, is deposited at GENT (HEC 10989). The specimen (Fig. 6.20) was compared to type material (including the lectotype specimen) deposited at LD (Fig. 6.22) and was found to agree well with the habit of the type material despite being slightly taller. Distance, maximum parsimony, maximum likelihood and Bayesian analyses resolved the *rbc*L sequence from this specimen as the most basal lineage in a well-supported clade of *Pterocladiella* species ([BP]=[D] 95, [P] 99, [L] , [Q] 73, [B] 100) in the *rbc*L phylogeny (Fig. 6.1).

Specimens examined: Type – Durban (Isipingo); Stephenson; 17 vii 1936; s.n. (loan no. 02/0170440, LD). Durban, The Bluff, Treasure Beach; Coppejans; 22 xi 1995; HEC 10989 (GENT).

Gelidium profundum Tronchin *nom. prov.*

Fig. 6.23-6.29

Description: Plants to 25 cm long, compound pinnate (to four orders) with untidy appearance. Axis flimsy, irregularly meandering and twisting. Branch length variable. Margins of lower axis and branches notched. Pinnae 0.5-3.5 (-6) mm long, (0.5-) 0.8-1.5 (-2) mm apart with slight basal constriction. Pinnae short (ca. 1-3 mm) along lower part of bearing branch, becoming longer and indeterminate along distal part. Branching angle broad (~ 78°). Apices rounded, apical cell dome-shaped. Holdfast stoloniferous, rhizoids terete producing brush-type secondary rhizoidal attachments. Axis cylindrical basally, compressed to flattened distally (to 1.5 mm wide). Nodes irregularly disposed on frond with frond thickness usually even either side of node. Vegetative structure consisting of outer cortex of small cells (5 μm), inner cortex of larger, angular cells (10 x 7 μm) and filamentous medulla (cells 13-25 μm in diameter). Rhizines concentrated in inner cortex. Fertile structures borne terminally or subterminally on ultimate pinnules. Tetrasporangia cruciate to decussate, cystocarps bilocular, spermatangia unknown.

Etymology: The specific epithet is derived from the Latin word *profundus* (adj.) meaning 'deep', since it has only been found to occur in deep (>-30 m) subtidal habitats.

Holotype: ET19 (BOL), Protea Banks (Northern Edge), KwaZulu-Natal Province, South Africa; Tronchin; 5 ii 2001; consists of two thallus fragments.

Ecology and Distribution: Occurring in deep-subtidal habitats (-30 to -35 m) on turf-covered reef outcrops. Lying sprawled over horizontal surfaces or hanging limply from walls, often in shaded areas. Only known from the type locality Protea Banks Reef, which lies 7.5 km offshore in the vicinity of Shelly Beach, southern KwaZulu-Natal coast, South Africa.

Specimens examined: Protea Banks (Northern Edge), KwaZulu-Natal Province, South Africa, -33m; Tronchin; 5 ii 2001; ET19 (BOL). Protea Banks (Northern Edge), KwaZulu-Natal Province, South Africa, -33m; Tronchin; 5 ii 2001; ET20 (BOL). Protea Banks, KwaZulu-Natal Province, South Africa, -34 m; Leliaert; 20 viii 1999; SP31 (BOL) - cystocarpic.

Plants are up to 25 cm long, compound pinnate and generally have an untidy appearance due to flimsy, irregularly meandering and twisting axes, variable branch lengths, and the notched margins (due to remnant basal branch stubs) of lower axes and branches (Fig. 6.23). Pinnae are short (ca. 1-3 mm) along the lower part of the bearing branch, becoming longer and indeterminate along the distal part. Their length generally ranges between 0.5-3.5 (-6) mm and they are (0.5-) 0.8-1.5 (-2) mm apart. There are up to four orders of branches which jut out at broad angles (~78°) with a slight basal constriction. Apices are rounded and have a dome-shaped apical cell that may be situated in an apical groove. The axis is cylindrical at the base becoming compressed and flattened distally, up to 1.5 mm wide. Nodes demarcating regrowth after injury are irregularly disposed on the frond and are evenly weighted, i.e. the frond thickness is generally even on either side of the node (Fig. 6.24c). Fronds are attached by a stoloniferous holdfast composed of terete rhizoids that produce attachment haptera consisting of elongate, aseptate cells (brush-type secondary rhizoidal attachments *sensu* Shimada *et al.*, 1999) when they come in contact with the substratum. Epiphytes include *Ptilophora diversifolia* (Suhr) Kützing, crustose corallines, sponges and hydroids.

In transverse sections of third order branches, the outer cortical cells are small (6 μm) and isodiametric (Fig. 6.25). The inner cortical cells are larger, angular (10 x 7 μm) and arranged in anticlinal filaments between which dense packets of rhizines occur. Rhizines are 3.5 μm in diameter and generally absent from the medulla. Medullary filaments are 13-25 μm in diameter.

Fertile structures are generally borne terminally on ultimate pinnules, sometimes subterminally to basally on branches (Fig. 6.26). Tetrasporangial sori occur in terminal branch swellings (Fig. 6.27) which are sometimes pear-shaped. Margins of tetrasporangial branchlets have subcortical bundles of rhizines which also occur throughout the medulla (Fig. 6.28). Tetrasporangia are cruciately to decussately divided. Cystocarps are bilocular with one ostiole per locule (Fig. 6.29). Spermatangia are unknown.

G. profundum is morphologically most closely related to *Gelidium pteridifolium*, but distinct differences do exist (Table 6.1). The mean branching angle in *Gelidium profundum* is 78° (std. dev. = 9.5) and in *G. pteridifolium* is 62° (std. dev. = 10.23). The Mann-Whitney U test performed on the branch angle data found a significant difference between the means ($Z = 14.489$; $p < 0.00$). Thalli of *G. pteridifolium* and *G. profundum* bear nodes on axes and branches, as do thalli of most Gelidiaceae taxa. Differences in the shape of these nodes have been noted and could provide an additional means of distinguishing between thalli of these species. Three types of nodes exist: a) distally weighted uneven nodes, where the axis is thicker just above the node (Fig. 6.24a); b) proximally weighted uneven nodes, where the axis is thicker just below the node (Fig. 6.24b); and c) evenly weighted nodes, where the axis is equally thick just either side of the node (Fig. 6.24c). Only five specimens of *G. profundum* have been observed with the objective of noting the shape of nodes and more specimens would need to be observed before results can be considered conclusive given the variation in node-type frequency observed in thirty specimens of *G. pteridifolium*. In these preliminary observations it appears that the nodes in *G. profundum* tend to be evenly weighted whereas in *G. pteridifolium* they tend to be either distally or proximally weighted uneven nodes (Fig. 6.30). Thalli of *G. pteridifolium* are wider and more rigid than those of *G. profundum* (Table 6.1), having relatively straight axes whereas there is a prevalence of bent and twisted axes in *G. profundum*.

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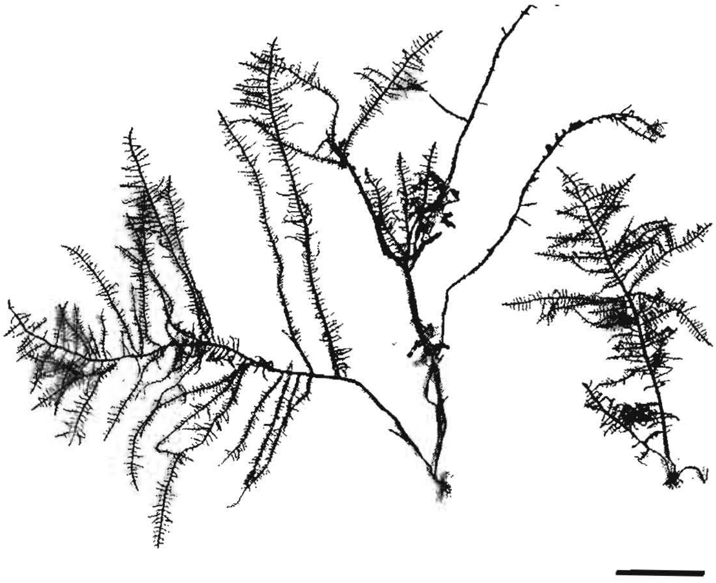

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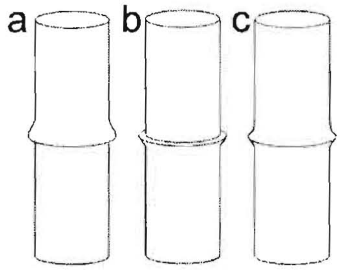

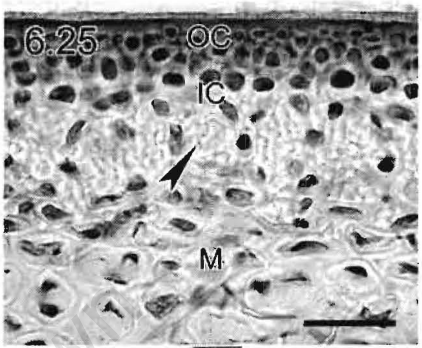

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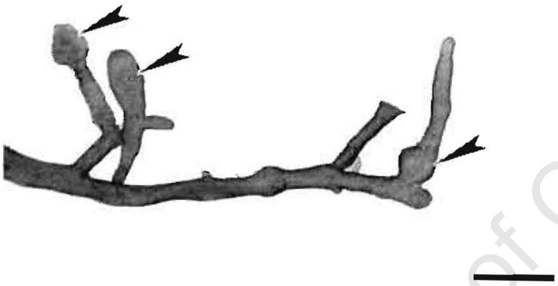

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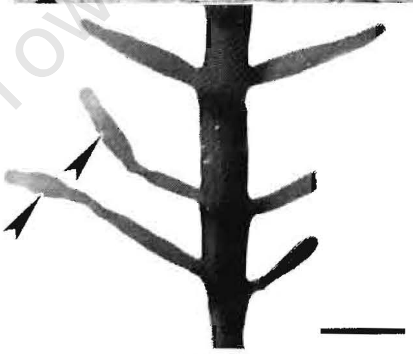

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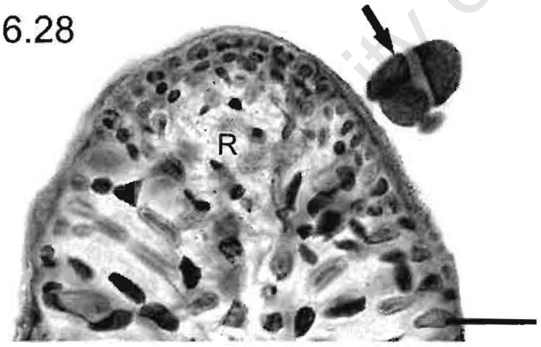

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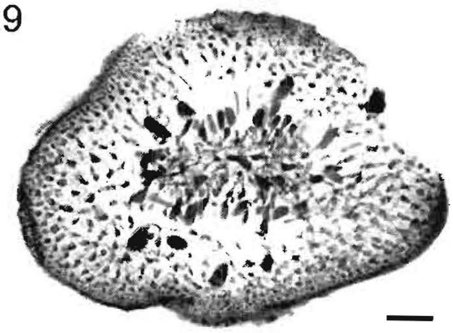

Fig. 6.23-6.29. *Gelidium profundum*. Fig. 6.23. Habit. Scale = 3 cm. Fig. 6.24. Schematic representation of three types of branch/axis nodes demarcating sites of regrowth after injury: a] distally weighted uneven node (axis thicker just above the node); b] proximally weighted uneven node (axis thicker just below the node; c] evenly weighted node (axis equally thick just either side of the node). *Gelidium profundum* tends to produce even nodes. Fig. 6.25. Transverse section of 1st order branch with rhizines (arrowhead) concentrated between anticlinal filaments of inner cortical cells. Outer cortex (OC), inner cortex (IC), medulla (M). Scale = 30 µm. Fig. 6.26. Cystocarps (arrowhead) borne terminally to basally on distal branches. Scale = 1 mm. Fig. 6.27. Tetrasporangial sori (arrowhead) borne terminally on pinnae. Scale = 1 mm. Fig. 6.28. Transverse section of tetrasporangial branchlet showing subcortical bundles of rhizines (R) at the branch margin and a decussately divided tetrasporangium that has been released (arrow). Scale = 30 µm. Fig. 6.29. Transverse section of bilocular cystocarp. Carposporangia borne either side of a central placental mass of nutritive cells and gonimoblastic filaments. Scale = 50 µm.

Table 6.1. Morphological characteristics of *Gelidium profundum*, *G. pteridifolium* and *G. abbottiorum*. Cell dimensions were measured from third order branches. Standard deviations from the mean are in brackets.

	Gelidium profundum	*Gelidium pteridifolium*	*Gelidium abbottiorum*
Max. plant height (cm)	25	30	28
Max. proximal axis width (mm)	1.1	1.4	0.8
Mean pinnule width (μm)	544 (± 53)	571 (± 90)	345 (± 43)
Mean pinnule thickness (μm)	201 (± 11)	178 (± 22)	225 (± 24)
Max. branch order	4	5	5
Compound pinnate	✓	✓	x
Branching regular	✓	✓	irregular
Pinnae straight	✓	✓	distal branches bend
Mean branching angle	78° (± 9.5)	62° (± 10.2)	90° (± 5.2)
Reflexed branches present	✓	x	✓
Branches flattened	✓	✓	terete to compressed
Proximal axes notched	✓	✓	x
Mean outer cortical cell diameter (μm)	5 (± 0.9)	6 (± 0.8)	6.5 (± 1)
Mean inner cortical cell length (μm)	10 (± 0.9)	10 (± 1.5)	11 (± 1.5)
Mean inner cortical cell width (μm)	7 (± 1.6)	8 (± 1.1)	8 (± 1.2)
Mean medullary cell length/diameter (μm)	14 (± 3.8)	12 (± 2.3)	13 (± 2.2)
Mean rhizine diameter (μm)	3.5 (± 0.5)	3.5 (± 0.5)	3.5 (± 0.6)

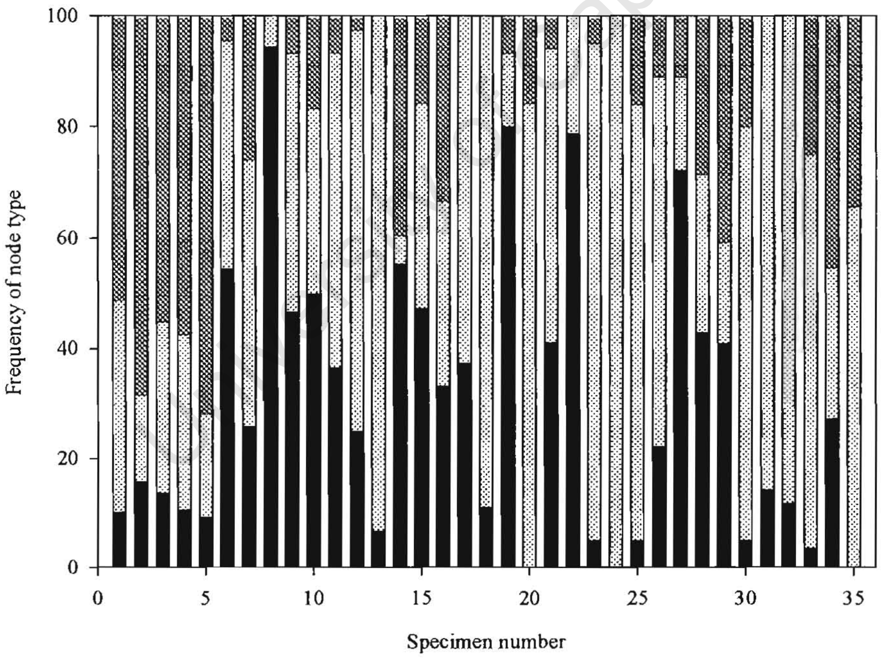

Fig. 6.30 – Frequency of node type in specimens of *Gelidium profundum* (specimens 1-5) and *Gelidium pteridifolium* (specimens 6-35). Node types: distally weighted uneven node (black), proximally weighted uneven node (light grey), evenly weighted node (dark grey).

The vertical and horizontal distributions of *G. profundum* and *G. pteridifolium* appear to be disjunct. According to current knowledge *G. pteridifolium* only occurs in the shallow subtidal (<-10 m) of inshore reefs, whereas *G. profundum* occurs much deeper at depths greater than -30 m and has only been reported from Protea Banks reef situated 7.5 km offshore.

All phylogenetic analyses of the *rbc*L sequence data resolved *G. abbottiorum* Norris, *G. pteridifolium* and *G. profundum* in the same strongly supported clade (Fig. 6.1). *G. pteridifolium* (Fig. 6.31) is morphologically distinct from *G. abbottiorum* (Fig. 6.32) and *G. profundum* (Table 6.1). The habit of *G. abbottiorum* is very tatty, sparsely and irregularly branched, whilst *G. pteridifolium* and *G. profundum* have a regular, compound pinnate (fern-like) appearance. *Gelidium abbottiorum* and *G. pteridifolium* have similar geographical distributions though *G. abbottiorum* is more widespread. *Gelidium abbottiorum* occurs in the intertidal and subtidal fringe of inshore reefs, overlapping with the vertical distribution of *G. pteridifolium* which occurs in the shallow subtidal. The two species can sometimes be found growing side-by-side in the subtidal fringe and can be easily distinguished at all times.

The sister relationships within the *rbc*L clade containing *G. abbottiorum*, *G. pteridifolium* and *G. profundum* could not be clearly resolved, though there appeared to be slightly more support for a sister relationship between *G. abbottiorum* and *G. pteridifolium*, contrary to morphology. The maximum likelihood analysis of the *rbc*L data resolved two trees, one showing a sister relationship between *G. abbottiorum* and *G. pteridifolium*, the other showing a sister relationship between *Gelidium pteridifolium* and *G. profundum* (a Shimodaira-Hasegawa test finding neither tree more likely). A distance and Bayesian analysis resolved a sister relationship between *G. abbottiorum* and *G. pteridifolium*, but branch support was poor ([BP] = [D] 63, [Q] 68, [B] 70). Of the nine minimal trees generated by the maximum parsimony analysis, two resolved *G. abbottiorum* and *G. pteridifolium* as sister taxa, four resolved the *G. pteridifolium* and *G. profundum* as sister taxa (poorly supported: [P] 61%), and the remaining three showed a polytomy between these three taxa. The sister relationships could not be clearly resolved because the *rbc*L sequences of these three taxa contain limited parsimony-informative data, having relatively few unambiguous character changes (0-2 changes). Furthermore, the sequence for *G. abbottiorum* is 296bp and 170bp shorter than the *G. pteridifolium* and *G. profundum* sequences respectively and this missing data may contain further differences that could clarify the relationships within this clade.

6.31

6.32

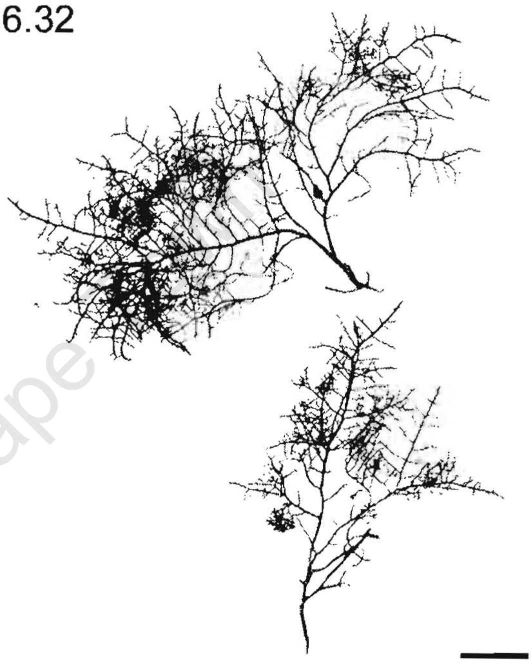

Fig. 6.31. Habit of *Gelidium pteridifolium* holotype at NU. Scale = 3 cm. Fig. 6.32. Habit of *Gelidium abbottiorum*. Scale = 2 cm.

*rbc*L Sequence divergence between all three taxa is similar, ranging between 1.1 and 1.2% (Table 6.2) which falls just out of the range of intraspecific sequence variability reported for some species in the Gelidiaceae (Freshwater and Rueness, 1994). Sequences of *G. abbottiorum* and *G. pteridifolium* differ by 1.1%, and both taxa are believed to be discrete species. Since *Gelidium profundum* differs by 1.14% and 1.2% from *G. abbottiorum* and *G. pteridifolium* respectively, it follows that *G. profundum* should be recognised as a separate species.

Table 6.2 – Matrix of *rbc*L sequence divergences (%).

		1	2	3
1	*Gelidium abbottiorum*	-		
2	*Gelidium pteridifolium*	1.095	-	
3	*Gelidium profundum*	1.144	1.219	-

Species Incertae

'Gelidium' sp.

Description: Plants up to 3.5 cm long. Holdfast stoloniferous and robust, stolons terete (up to 1 mm in diameter) and often longer than the upright axes which develop at irregular intervals along stolons. Upright axes and decumbent lateral branches can reattach to substratum and give rise to new uprights. Prostrate branches formed in this manner are flattened to compressed. Holdfast produces brush-type secondary rhizoidal attachments. Uprights flattened, 1.5-2 (-2.8) mm wide, and 400-500 µm thick. Up to three orders of distichous branching. Branching irregular to pinnate, opposite to subopposite. Branches basally constricted, emarginate when young, lanceolate to ligulate when old. Nodes common on prostrate stolons. Branches anastomose frequently. Outer cortical cells are ca. 4 x 8 µm, inner cortical cells 9.5 x 10 µm, rhizines 2.5-4 µm in diameter, and medullary cells 8-12 µm in diameter. Cell dimensions greater in the stolons. Only vegetative thalli known.

Ecology and Distribution: Plants known only from the KwaZulu-Natal coastline, from three offshore reefs in the Sodwana area, i.e. Four Buoy, Seven Mile and Nine Mile reefs. Growing in sandy subtidal habitats to -20 m at the bottom of crevices or on ledges of coral reef, generally not occurring on vertical walls. The prostrate system of the plants are normally covered in sand with only the erect uprights visible.

Specimens examined: Nine Mile Reef, Sodwana, KwaZulu Natal Province, South Africa; Freshwater; 10 ii 2001; coll. no. P25 – wet preserved. Four Buoy Reef, KwaZulu Natal Province, South Africa, -15 to -17 m; Tronchin; 30 iii 2002; S26 (BOL) – silica gel dried. Seven Mile Reef, KwaZulu Natal Province, South Africa, -15 to -20 m; Tronchin; 4 iv 2002; P35 (BOL) – wet preserved.

Plants are up to 3.5 cm tall with a stoloniferous holdfast of robust, terete stolons (up to 1 mm in diameter) that are often longer than the upright axes which develop at irregular intervals along the stolons (Fig. 6.33). Axes and decumbent lateral branches can reattach to the substratum and form part of the prostrate system giving rise to new uprights. Prostrate branches formed in this manner are flattened to compressed. Prostrate branches with tapered ends are produced from the stolons and the most proximal parts of upright axes. These produce brush-type secondary rhizoidal attachments *sensu* Shimada *et al.* (1999).

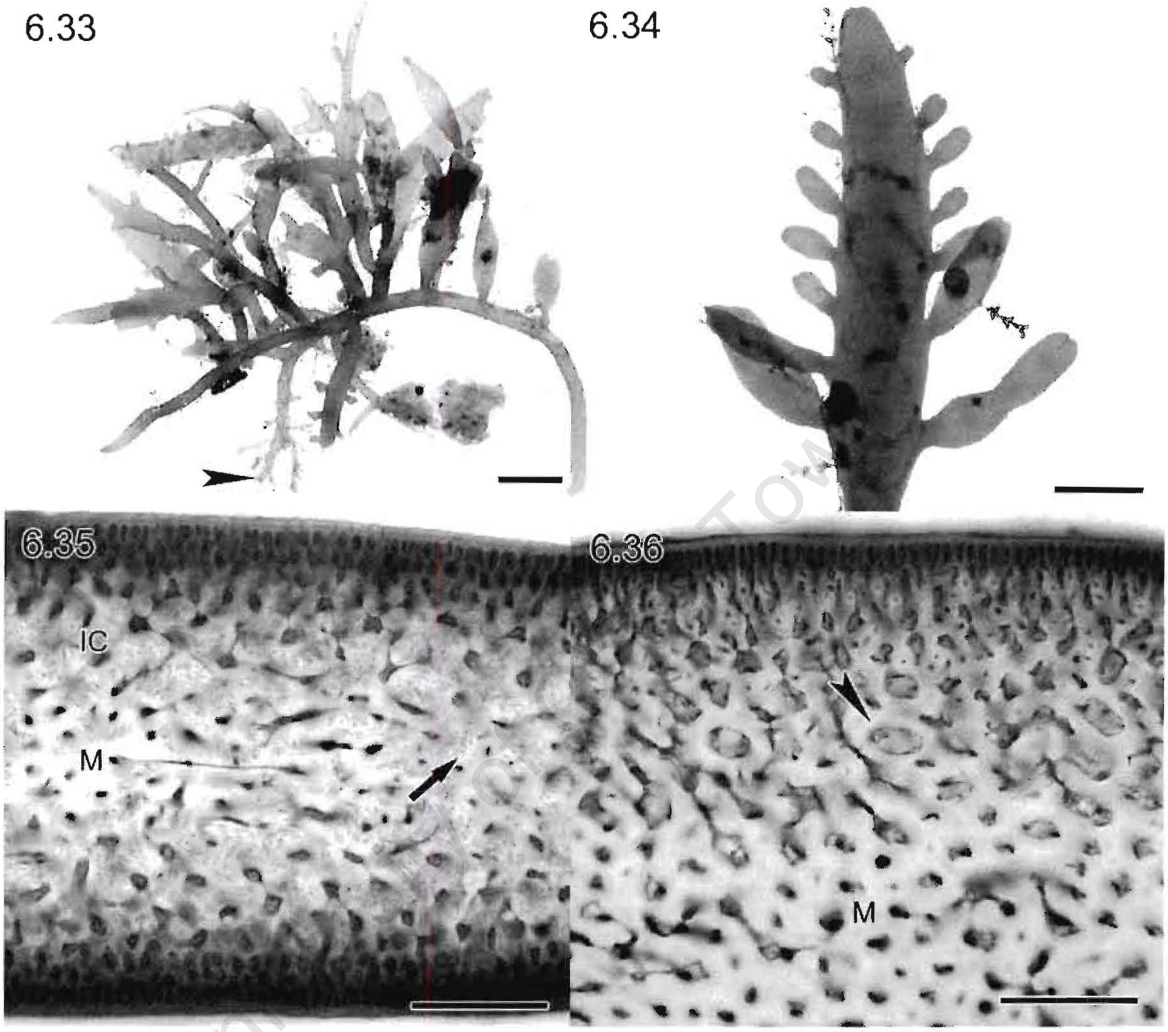

Fig. 6.33-6.36. *Gelidium* sp. from Sodwana. Fig. 6.33. Habit. A robust stolon gives rise to uprights at irregular intervals and rhizoidal branches producing brush-type secondary rhizoidal attachments (arrowhead). Scale = 3 mm. Fig. 6.34. Pinnately branched upright axis. Scale = 2 mm. Fig. 6.35. Transverse section of flattened upright. Rhizines (arrow) are concentrated in the inner cortex (IC) and are interspersed between medullary filaments (M). Scale = 50 µm. Fig. 6.36. Transverse section of stolon. Some inner cortical cells (arrowhead) can be larger than the medullary filaments (M) in diameter. Scale = 50 µm.

Uprights are terete basally becoming flattened within 2 mm of the base, and are generally 1.5-2 (-2.8) mm wide, and 400-500 µm thick. Up to three orders of branches are produced. These are initially simple with entire margins becoming distichously branched. Branching is irregular to pinnate, opposite to subopposite (Fig. 6.34). Branches bear a basal constriction. Pinnae are somewhat heart-shaped when young due to their emarginate apices, and are lanceolate to ligulate when old. Nodes are common on prostrate stolons and truncated, proliferating ends on uprights and lateral branches demarcate sites of injury. Apical cells are borne in the apical groove. Branches anastomose frequently. Anastomoses can be short cylinders (1-2 mm long) or discoid fusion points. Anastomosing usually involves prostrate branches.

Outer cortical cells are ca. 4 x 8 µm (up to 5 x 9), inner cortical cells are 9.5 x 10 µm, rhizines 2.5-4 µm in diameter, and medullary cells 8-12 µm in diameter (Fig. 6.35). Cells dimensions are greater in the stolons, outer cortical cells reaching up to 10 µm in length, inner cortical cells up to 27 µm, rhizines up to 4.5 µm, and medullary cells 19 µm in diameter in transverse sectional view (Fig. 6.36). Outer cortical cells are elongated in transverse section and anticlinally arranged. Rhizines are distributed throughout the inner cortex and may be scattered in the medulla. Thick pit connections are present between cells in the medulla and the inner cortex. In the stolons, some inner cortical cells may be larger in diameter than the medullary cells. Medullary cells are thick-walled.

Gelidium sp. was resolved basal to a clade containing *Gelidium*, *Ptilophora* Kützing, *Capreolia* Guiry *et* Womersley and *Acanthopeltis* Okamura clades in distance and maximum likelihood analyses. In the Bayesian phylogeny it was basal to the aforementioned clades, in a clade with *Gelidiella acerosa* (Forsskål) Feldmann *et* Hamel, though separated from the latter by long branch-length. The taxon was resolved as the second most basal lineage in the maximum parsimony tree (Fig. 6.1). Little to no branch support was found for these basal relationships; however, it is clear that *Gelidium* sp. is a member of the Gelidiaceae, though molecularly distinct from all the genera in the family, and distinct from *Gelidiella* Feldmann *et* Hamel, also in that it produces rhizines which are more or less absent in *Gelidiella*. Based on the *rbc*L data, *Gelidium* sp. could be recognised as a new genus in the Gelidiaceae, but such distinction should ideally be accompanied by a consistent morphological difference in female reproductive and cystocarp development.

All specimens collected to date are vegetative and until such time as female reproductive material is collected and observed, the taxon should be treated as an undescribed species of *Gelidium*, the genus to which it appears most closely related morphologically. The prominent and robust holdfast producing brush-type secondary rhizoidal attachments is the most distinctive feature of this taxon, resembling the stoloniferous holdfast of large frondose *Gelidium* species such as *G. pteridifolium*, *G. capense* and *G. abbottiorum*. The taxon is similar to *Gelidiella* in the production of anastomosing branches and similar to *Ptilophora* in vegetative structure due to the occasional production of large inner cortical cells that are greater in diameter than the medullary filaments. The upright axes are akin to those of *Pterocladiella caerulescens* in that they are flattened, lanceolate and pinnately branched, though sometimes considerably broader and more robust.

Pterocladiella caerulescens species complex

Two morphologically distinct taxa were collected from the Sodwana Bay area in northern KZN and have not yet been reported for South Africa. Both taxa are closely related morphologically and molecularly to *Pterocladiella caerulescens*, a species originally described from New Caledonia (Kützing, 1868), and since reported from the Caribbean, the tropical West Pacific and the Hawaiian Isles (Abbott 1999, Felicini and Perrone 1994, Payri *et al.* 2000, Price and Scott 1992, Santelices 1998, Shimada and Masuda 2000). The two South African taxa, referred to here as *Pterocladiella 'caerulescens'* and *Pterocladiella* sp., are described below.

Pterocladiella 'caerulescens'

Description: Plants up to 3 cm long, holdfast stoloniferous, peg-like secondary rhizoidal attachments produced. Uprights lanceolate, subcylindrical proximally, flattened and ligulate distally, 1-1.5 mm wide, 100-180 µm thick, to bipinnate. Branching alternate to opposite, 0.5-5 mm apart. Pinnae oblanceolate to ligulate and lanceolate. Apices obtuse, often emarginated. Apical cell occasionally protuberant. In transverse section outer cortical cells anticlinally elongate to angular, ca. 10 x 6 µm, length/width ratio 1.68; inner cortical cells angular, ca. 12 x 9 µm, length/width ratio 1.39; rhizines concentrated in medulla, 4 µm in diameter; medullary filaments 11-17 µm in diameter. Cystocarps produced subterminally.

tetrasporangial sori terminally, on axes and ultimate branches. Cystocarps unilocular, placental core centrally positioned in the mature cystocarp, carposporangia not produced on side facing cystocarp floor. Pericarp elevated on one side of branch, ostioles lack peristome.

Ecology and distribution: Only known from 2 Mile Reef, Sodwana growing on coral reef outcrops at depths of up to -12 m.

Specimens examined: 2 Mile Reef, Sodwana, KwaZulu-Natal Province, South Africa; Tronchin; 10 ii 2001; P17 (BOL).

Plants are up to 3 cm long and are attached by a stoloniferous holdfast (Fig. 6.37, 6.38) with compressed stolons (up to 450 x 340 μm in transverse section), and terete to compressed rhizoidal branches producing peg-like secondary rhizoidal attachments (Fig. 6.39). Uprights are lanceolate, subcylindrical proximally, flattened and ligulate distally, 1-1.5 mm wide, 100-180 μm thick and up to twice pinnate. Branching is alternate to opposite, 0.5-5 mm apart. Pinnae are 0.45-1 mm wide and up to 8 mm long. Pinnae are oblanceolate when young, appearing ligulate and lanceolate when mature with obtuse, often emarginated, apices. The apical cell is dome-shaped and occasionally protuberant.

In transverse section, the outer cortical cells are anticlinally elongated and sometimes angular, 10 x 6 μm on average with a length/width ratio of 1.68 (Fig. 6.40). Inner cortical cells are angular, 12 x 9 μm on average with a length/width ration of 1.39. Rhizines are mostly situated in the medulla, scattered in the inner cortex and ~4 μm in diameter. Medullary filaments are 11-17 μm in diameter.

Fertile structures are produced on distal ends of axes and ultimate branches, subterminally in the case of cystocarps (Fig. 6.37) and terminally in the case of tetrasporangial sori (Fig. 6.38). Cystocarps are unilocular (Fig. 6.41), the pericarp is elevated only on one of the blade surfaces and ostioles lack a peristome. The placental core of nutritive cells and gonimoblastic filaments surrounding the axial cell row is centrally positioned in the mature cystocarp. Carposporangia are produced around this core except for the side facing the floor of the cystocarp.

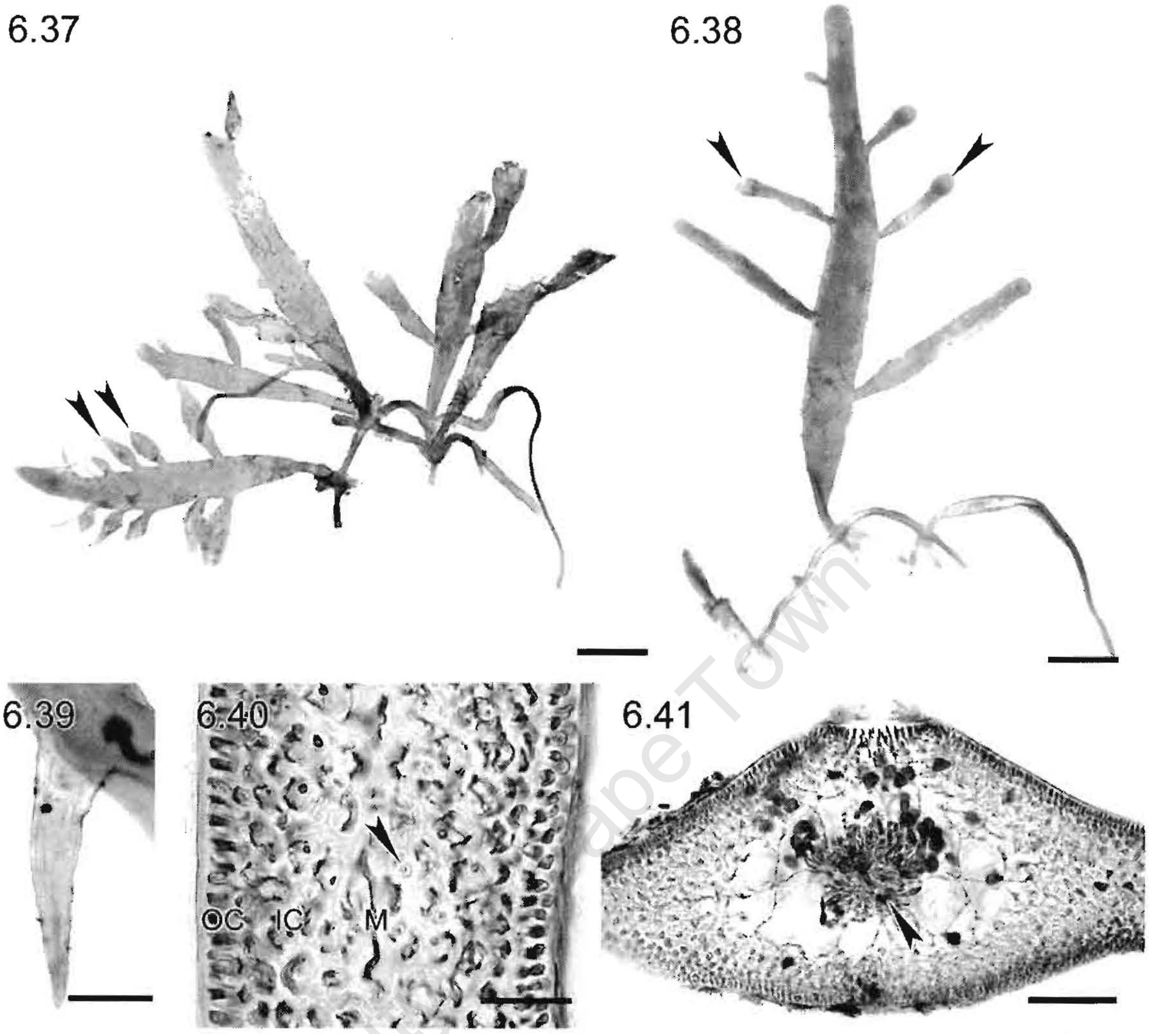

Fig. 6.37-6.41. *Pterocladiella 'caerulescens'* from Sodwana. Fig. 6.37. Cystocarpic habit. Cystocarps occur subterminally on axes and branches (arrowhead). Scale = 2 mm. Fig. 6.38. Tetrasporic habit. Tetrasporangial sori occur terminally in slightly broadened branch ends (arrowhead). Scale = 2 mm. Fig. 6.39. Peg-like secondary rhizoidal attachment. Scale = 200 µm. Fig. 6.40. Transverse section of flattened upright with rhizines (arrowhead) occurring in the medulla. Outer cortex (OC); inner cortex (IC); medulla (M). Scale = 30 µm. Fig. 6.41. Transverse section of uniloculate cystocarp with centrally positioned placental core (arrowhead). Carpospores not borne on the side of placental core facing cystocarp floor. Scale = 100 µm.

Pterocladiella sp.

Diagnosis: Plants up to 5 cm tall, holdfast stoloniferous, producing peg-like secondary rhizoidal attachments. Uprights lanceolate, flattened, 0.7-1.8 mm wide, 100-160 µm thick. Uprights sometimes very narrow in flattened parts (~0.3 mm) with terete distal parts, or compressed to terete throughout. Branching irregular to three orders. Branches 0.1-3 mm apart, 80-700 µm wide, up to 35 mm long, linear to lanceolate, usually basally constricted. Axes and branches become narrow and terete (~80-180 µm in diameter) distally, alternately to oppositely branched, when contact with substratum is made. Apices obtuse to acute, apical cell protuberant. In transverse section, outer cortical cells anticlinally elongated to angular, ca. 8 x 5 µm, length/width ratio 1.59; inner cortical cells angular, 13 x 9.5 µm, length/width ratio 1.38; rhizines ~6 µm, abundant medulla; medullary filaments 11-26 µm in diameter. Cystocarps produced subterminally on axes and ultimate branches. Cystocarp unilocular, placental core not attached to the cystocarp wall, carposporangia developing on all sides of core. Pericarp elevated on one of the branch surfaces, ostioles lack peristome. Gametophytic plants monoecious, spermatangial sori occurring on cystocarpic branches.

Ecology and distribution: Occurring on 9 Mile and 2 Mile reefs, Sodwana, -10 to -12 m deep. Growing attached to coral fragments at the bottom of sandy gullies. The prostrate system is covered by sand with only the uprights extending above the sandy substrate. Axes and branches often decumbent and reattached to the substrate via terete, branched distal parts.

Specimens examined: 9 Mile Reef, Sodwana, KwaZulu-Natal Province, South Africa; Tronchin; 12 ii 2001; P24 (BOL). 2 Mile reef, Sodwana, KwaZulu-Natal Province, South Africa; Tronchin; 10 ii 2001; P34 (BOL).

Plants are 3.5 (-5) cm long and attached by a stoloniferous holdfast (Fig. 6.42, 6.43. Stolons are compressed, (130-) 300 x 590 µm in diameter, branched and issue peg-like secondary rhizoidal attachments (Fig. 6.44). Uprights are lanceolate, compressed basally becoming quickly flattened and are 0.7-1.8 mm wide and 100-160 µm thick. Uprights may sometimes be very narrow in flattened parts (~0.3 mm) with terete distal parts, or compressed to terete throughout. There are up to three orders of irregular marginal branching. Branches are 0.1-3 mm apart, 80-700 µm wide, usually up to 8 (-35) mm long, linear to lanceolate and usually basally constricted (Fig. 6.45). When axes and branches come in contact with the substratum they can become narrow and terete (~80-180 µm in diameter), producing alternately to oppositely arranged branches subtended at right angles in distal parts (Fig. 6.42). These terete distal branches can sometimes be numerous and intertwined forming bundles (Fig. 6.43). Axes and branches have obtuse to acute apices bearing a protuberant apical cell.

In transverse section, the outer cortical cells are anticlinally elongate, sometimes angular and 8 x 5 μm on average with a length/width ratio of 1.59 (Fig. 6.46). Inner cortical cells are angular and 13 x 9.5 μm on average with a length/width ratio of 1.38. Rhizines are abundant in the medulla and ~6 μm in diameter. Medullary filaments are thick-walled and 11-26 μm in diameter.

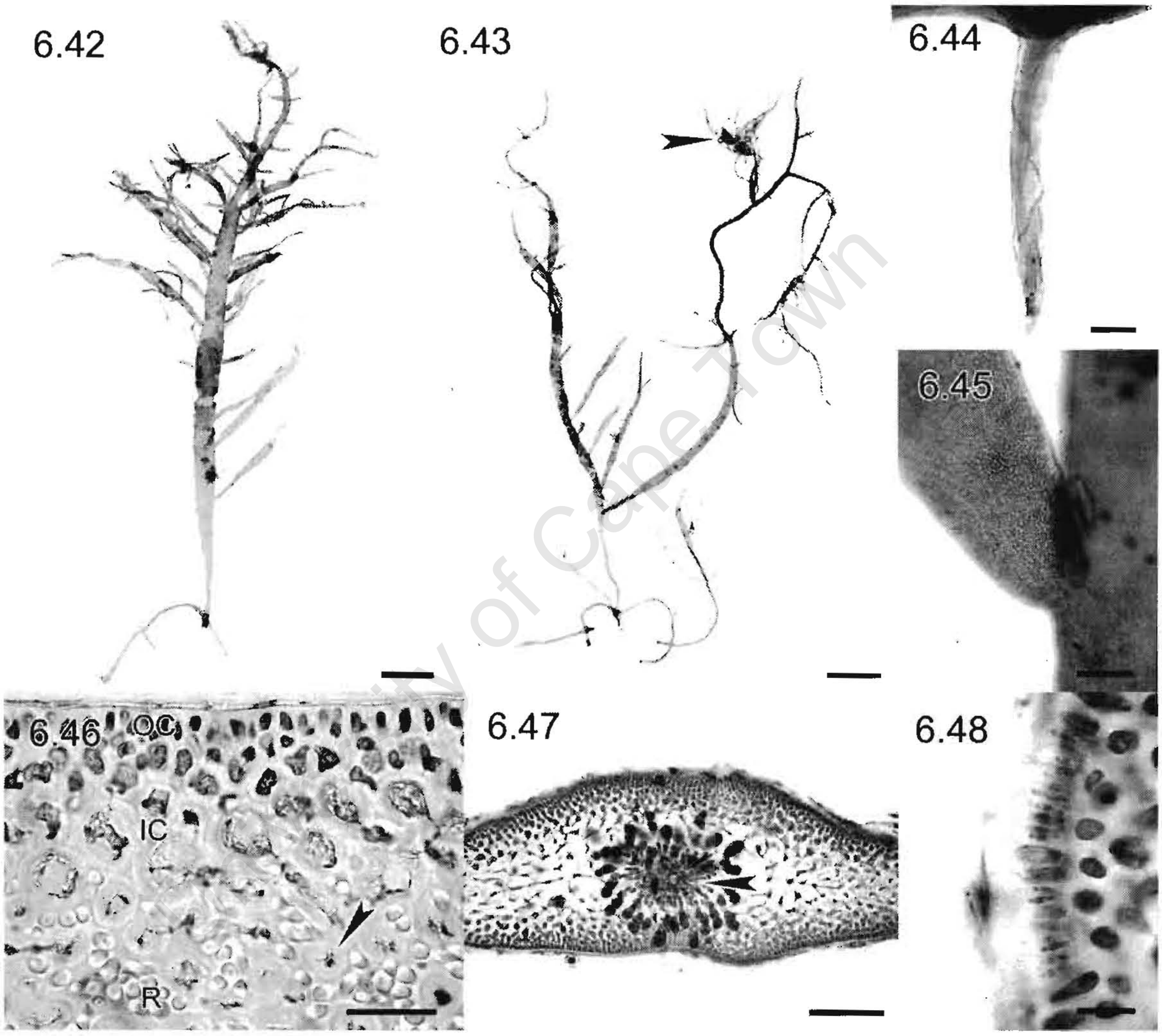

Fig. 6.42-6.48. *Pterocladiella* sp. from Sodwana. Fig. 6.42. Habit of plant with broad, lanceolate axes. Distal parts are terete. Scale = 3 mm. Fig. 6.43. Habit of plant with narrow, sparsely branched axes and terete distal parts. Bundles of terete branches are occasionally borne distally (arrowhead). Scale = 3 mm. Fig. 6.44. Peg-like secondary rhizoidal attachment. Scale = 200 μm. Fig. 6.45. Basal branch constriction of primary branch. Scale = 100 μm. Fig. 6.46 Transverse section of flattened upright. Rhizines (R) are abundant between medullary filaments (arrowhead). Outer cortex (OC), inner cortex (IC). Scale = 30 μm. Fig. 6.47. Transverse section of uniloculate cystocarp. The placental core (arrow) is centrally positioned in the locule bearing carpospores on all sides. Scale = 100 μm. Fig. 6.48. Transversely divided spermatangial initials forming a single-layered outer cortex. Scale = 10 μm.

Cystocarps are subterminal and unilocular. The placental core of nutritive cells and gonimoblast filaments surrounding the axial cell row is not attached to the cystocarp wall, but lies centrally positioned in the locule (Fig. 6.47). Carposporangia develop on all sides of the placental core. The pericarp is elevated from one of the blade surfaces. Ostioles do not have a peristome. Gametophytic plants are monoecious, elongate spermatangial initials having been found towards the margins of cystocarpic branches in narrow patches (Fig. 6.48). Tetrasporic plants are unknown.

Pterocladiella 'caerulescens' has been so named since it matches well with various descriptions and illustrations of *P. caerulescens* from the Pacific (Felicini and Perrone, 1994, Price and Scott 1992, Santelices 1976, 1977, 1978, 1998, Santelices and Stewart 1985, Shimada and Masuda 2000)(Table 6.3). Given that carposporangia are not produced on all sides of the cystocarp placental core (being absent on the side facing the cystocarp floor) and that the pericarp is elevated on only one blade surface, it is suspected that the placental core is secondarily detached in the mature cystocarp, in which case, the cystocarp morphology of *P. 'caerulescens'* would agree with that of *Pterocladiella* (Santelices 1998, Santelices and Hommersand 1997, Shimada and Masuda 2000, Santelices and Stewart 1985, Felicini and Perrone 1994). Cystocarpic material was limited in the collection of *P. 'caerulescens'*. Young cystocarps need to be sectioned in order to determine whether the placental core is secondarily detached from the cystocarp floor.

Pterocladiella sp. bears similarities to *P. caerulescens*, but has some distinctly different morphological characters. This taxon is morphologically variable: some plants have uprights that approximate some forms of *P. caerulescens* from the Hawaiian Islands (fig. 11 in Santelices 1976, fig. 2a in Santelices 1978) with respect to the slenderness of the axes and the frequency and proximity of lateral branches, whilst other plants have uprights that are markedly narrower (being mostly compressed to terete) than those of *P. caerulescens*. *Pterocladiella* sp. can be distinguished from *P. caerulescens* by its narrower blade and the production of terete distal parts. Fronds of *P. caerulescens* have not been known to become decumbent and reattach to the substrate producing terete distal parts. Cystocarps of *Pterocladiella* sp. have a centrally positioned placental core bearing carposporangia on all sides, a morphology that allies it with *P. beachiae* (published as *P. beachii*, but the gender of this specific epithet is incorrect as the person it honours is female) rather than *P. caerulescens*. *Pterocladiella beachiae* was recently described from Costa Rica and reported to have a unique cystocarp structure in which the placental core never attaches to the cystocarp wall throughout the maturation of the cystocarp, and produces carposporangia on all sides. This species falls within the range of variation for most morphological characters described for *P. caerulescens* in the Pacific (Table 6.3), but differs in the morphology of the cystocarp and the fact that branches are basally constricted compared to the more basally tapered branches of *P. caerulescens* (Freshwater and Thomas, 2001). *Pterocladiella* sp. gametophytes were found to be monoecious as has been previously reported for species of this genus (Santelices and Hommersand 1997, Thomas and Freshwater 2001, Shimada and Masuda 2000).

Maximum parsimony, maximum likelihood, distance and Bayesian analyses resolved *P. 'caerulescens'* (South Africa), *P. beachiae* (Costa Rica) and *P. caerulescens* (Hawaii) in a well supported clade ([BP] = [P] 83, [D] 80, [B] 98)(Fig. 6.1). Maximum likelihood and distance analyses resolved *P. 'caerulescens'* sister to *P. caerulescens* with no bootstrap support [BP] = [Q] 54, [D] 56), whilst maximum parsimony and Bayesian analyses resolved *P. 'caerulescens'* as sister to *P. beachiae*, with moderate bootstrap support however ([BP] = [P] 73, [B] 91). Three specimens of *Pterocladiella* sp. were resolved in a separate clade sister to the abovementioned taxa. *rbc*L Sequence divergences between multiple sequences of *Pterocladiella* sp. and multiple sequences of *P. caerulescens* ranged from 0.0-0.3%, whilst divergences between sequences of these two taxa and *P. 'caerulescens'* and *P. beachiae* ranged from 1.9-3.4% (Table 6.4). Thus, sequence divergences suggest that there are four distinct species since the divergences between taxa falls out of the range of intraspecific variability usually observed in the Gelidiaceae. All phylogenetic analyses resolved the four taxa in the same strongly supported clade ([BP] = [P] 100, [D] 100, [Q] 85, [B] 100).

Table 6.3. Morphological characteristics of four taxa in the *Pterocladiella caerulescens* species complex. Data for *P. caerulescens* was adapted from Felicini and Perrone (1994), Kützing (1868), Santelices (1977), Santelices and Stewart (1985), and Shimada and Masuda (2000). Data for *P. beachiae* was adapted from Thomas and Freshwater (2001).

	Pterocladiella caerulescens	*Pterocladiella 'caerulescens'*	*Pterocladiella beachiae*	*Pterocladiella* sp.
Axis height (cm)	3.3 (-7)	3	2.5	3.5 (-5)
Axis width (mm)	1.3 -1.8	1-1.5	0.6-2	0.3-1.8
Axis thickness (µm)	100-175	100-180	110	100-160
Branching	Simple alternate to quadripinnate	Pinnate (to 2 orders), opposite to alternate	Pinnate to alternate, up to 3 orders	Irregular, alternate to opposite distally
Axis shape	Lanceolate, ligulate	Lanceolate, ligulate	Lanceolate, ligulate	Lanceolate, ligulate, terete distally
Branch shape	Oblanceolate to ligulate and lanceolate	Oblanceolate to ligulate and lanceolate	Oblanceolate to ligulate and basally constricted	Linear to lanceolate and basally constricted
Apex shape	Obtuse to emarginate	Obtuse to emarginate	Obtuse	Obtuse
Position of fertile tissue	Apex of axes and branches	Apex of axes and branches	Apex of axes and branches	Apex of axes and branches
Holdfast type	Stoloniferous, peg-like rhizoidal attachments	Stoloniferous, peg-like rhizoidal attachments	Stoloniferous, peg-like rhizoidal attachments	Stoloniferous, peg-like rhizoidal attachments
Stolon compressed	✓	✓	✓	✓
Stolon diameter (µm)	(140-) 200-350	200-450	210-380	130-310
No. of cortical cell layers*	3-4	3-4	3-4	3-4
Outer cortical cell size (µm)*	4-8 x 3-4		6-10 x 2.5-4	
Max. inner cortical cell diameter (µm)*	15		13	
Medullary cell diameter (µm)*	10-20		6-13	
Location of rhizines*	Abundant in medulla	Abundant in medulla	Abundant in medulla	Abundant in medulla
Cystocarp location	Subterminal on axis and branches	Subterminal on axis and branches	Subterminal on axis and branches	Subterminal on axis and branches
Peristome present	x	x	x	x
Pericarp elevated on one blade surface	✓	✓	✓	✓

* Measured from cells in transverse section of erect branch. Cell proportions, stolon dimensions and the thickness of the upright of *P. beachiae* were measured from figures 19, 21 and 22 respectively, of Thomas and Freshwater (2001); figures 16 and 18 were used to calculate the width of the axis.

Table 6.4. Matrix of Tamura-Nei calculated divergences (%) between *rbc*L sequences from specimens of *Pterocladiella caerulescens* from Hawaii, *P. beachiae* from Costa Rica and *P. 'caerulescens'* and *Pterocladiella* sp. from South Africa.

		1	2	3	4	5	6	7
1	*Pterocladiella 'caerulescens'*	-						
2	*Pterocladiella* sp.	3.447	-					
3	*Pterocladiella* sp.	3.071	0.112	-				
4	*Pterocladiella* sp.	3.071	0.112	0.000	-			
5	*Pterocladiella caerulescens*	2.707	2.259	2.722	2.722	-		
6	*Pterocladiella caerulescens*	2.608	2.369	2.786	2.786	0.288	-	
7	*Pterocladiella beachiae*	1.877	2.012	2.500	2.500	2.148	2.286	-

It appears that *P. caerulescens* represent a species complex. Of the four molecularly distinct taxa discussed here that fit a broad description of *P. caerulescens*, three (*P. 'caerulescens'*, *P. caerulescens* and *P. beachiae*) have indistinct vegetative thalli and two (*Pterocladiella* sp. and *P. beachiae*) have a different cystocarp morphology to that of *P. caerulescens*. Further population and subpopulation sampling is necessary to further elucidate sister relationships between *P. caerulescens* populations worldwide, to determine sequence divergences between and within these populations, to determine the consistency of observed morphological differences, and to find additional morphological characters to support the various clades within this species complex.

GENERAL DISCUSSION

There are fourteen turf-form Gelidiaceae species in South Africa, nine of which belong to *Gelidium* (Table 6.5) and five of which belong to *Pterocladiella* (Table 6.3, 6.5). All are distinguishable by various distinctive external and vegetative morphological characters, though difficulty in distinguishing between thalli of *Gelidium isabelae* and the intertidal form of *G. reptans* may be experienced. The rhizome of *G. isabelae* is usually half the diameter of that of *G. reptans* and the erect axes are slightly smaller in stature and stipitate. *Gelidium isabelae* also shows no signs of the constriction or twisting of erect axes as is often found to occur in *G. reptans*. There is still enormous scope for systematic research on the intertidal and subtidal turf-form Gelidiaceae. In particular, knowledge of reproductive structures is incomplete in some species. *Gelidium applanatum* Stegenga, Bolton *et* Anderson, *G. arenarium*, *G. minusculum* and *Pterocladiella caloglossoides*

(Howe) Santelices have yet to be sequenced. Efforts were made to document and discuss all taxa of South African Gelidiaceae that could be supported by molecular and morphological data, but a number of specimens still exist in the various algal collections around the country that are potentially new species or new records, but could not be considered in this study for practical reasons.

Six new species/records (*Gelidium sp., G. profundum, Ptilophora coppejansii* Tronchin, *P. leliaertii* Tronchin, *Pterocladiella sp., P. 'caerulescens'*) were found on Protea Banks and three offshore reefs in the Sodwana Bay area. Some have large frondose habits and others are fairly ubiquitous in their respective habitats. Except for two drift specimens of *Ptilophora leliaertii* at BOL, no record was found of the remaining five species at any of the major algal collections in the country (BOL, GRA and NU). Thus, it would seem that the seaweeds of these offshore reefs have been poorly sampled, despite both locations being popular dive sites and readily accessible by numerous charter companies. Further research on collections from these reefs and a host of other less accessible offshore reefs along the South African east coast would undoubtedly yield many more new species and records.

Table 6.5. Morphological characters of small (<5 cm) South African Gelidiaceae species.

Character	***Gelidium applanatum***	***Gelidium micropterum***	***Gelidium foliaceum***	***Gelidium declerckianum***	***Gelidium arenarium***	***Gelidium crinale***
Height	<5 mm	5 cm	<10 mm	2-15 mm	0.5-1.5 (-30) mm	<5 cm
Secondary rhizoidal attachment	bundles of multicellular rhizoids or discoid	discoid	discoid	brush-like	brush-like	discoid
Branching pattern	irregular, lobed	simple to tripinnate, pinnae closely spaced	simple to irregularly pinnate	up to 5 orders, irregular, distichous	simple to irregular, opposite distally	sparse, irregular, branches often twisted together and restricted to apical half, usually appearing as dichotomies
Prostrate branch shape	foliaceous	compresses, sometimes pinnately-lobed	cylindrical to compressed	cylindrical	cylindrical	cylindrical
Erect branch Shape	simple, ovate to cuneate	flattened, lateral lobes twisted and contorted	undulate, caulescent lower region, irregularly palmate distally, margins entire to eroded to crispate	crisped, irregularly curled and twisted, blunt to tripartite apex	cylindrical to compressed distally, apices often tripartite	cylindrical basally, flattened above, broadest at origin of branches
Erect branch Dimensions	1-1.5 mm wide, <100 μm thick	<1.2 mm wide, <150 μm thick	<1.5 mm wide, <200 μm thick	<600 μm wide distally, <140 μm thick	250 μm diameter	<50 μm thick, <500 μm wide at branch origin
Rhizine number and distribution	rhizines few, situated in medulla	rhizines confined to medulla	in medulla	situated in inner cortex and medulla	in outer medulla and inner cortex	few in medulla
Appearance of medulla in cross-section	rounded cells	rounded cells	stellate cells	rounded cells	distinct axial cell surrounded by ca. 5 first order cells	rounded cells
Apical cell character	sunken in emarginate apex	indistinct or sunken in emarginate apex	sunken in emarginate apex	pronounced	pronounced	pronounced
Tetrasporangial sori	unknown	not found	not found	initially acropetally developed, sori subterminal	spatulate, borne terminally	irregularly shaped, in upper compressed branches
Bisporangial sori	unknown	transversely divided in ultimate/ penultimate, rounded/ ellipsoidal branchlets	mono- or bisporangia in ill-defined sori in proliferous bladelets	not found	not found	not found
Cystocarps	bilocular, borne subterminally on lobes	unknown	bilocular, in marginal lobes	unknown	unknown	bilocular, borne subterminally

Table 6.5 (cont.). Morphological characters of small (<5 cm) South African Gelidiaceae species.

Character	***Gelidium minusculum***	***Gelidium isabelae***	***Gelidium reptans***	***Pterocladiella caespitosa***	***Pterocladiella bartlettii***	***Pterocladiella caloglossoides***
Height	<2 mm	1-5 mm	1-25 mm	usual 10-30 mm	<13 cm	up to 3mm
Secondary rhizoidal attachment	brush-like	discoid	brush-like	peg-like	discoid	peg-like
Branching pattern	unbranched	usually unbranched, very seldom distichously branched	simple ligulate, to pinnate	somewhat umbellate proximally, simple to distichous distally	irregular, sparse (young), bilateral series of branchlets and pinnate branching (mature)	mostly unbranched, up to 5 erect axes issuing from same point on prostrate branch
Prostrate branch shape	cylindrical	cylindrical, <100 µm in diameter	cylindrical, ca. 200 µm in diameter	cylindrical	cylindrical to compressed	cylindrical to compressed
Erect branch Shape	simple, flattened, lanceolate	flat & stipitate; ligulate to spatulate or lanceolate, obtuse apex	flattened, sometimes marginally constricted and twisted when <1 cm	compressed, to flattened, usually has bilateral series of bladelets distally	cylindrical to compressed to flattened, linear lanceolate to pinnate	compressed, simple, reflexed or repent
Erect branch Dimensions	<500 µm wide, <400 µm thick	<500 µm, <150 µm thick	0.4-1mm wide	0.3-1 mm wide, <200 µm thick	0.1-0.25 mm wide, 135-160 µm thick	<3 mm long, 100-400 µm wide
Rhizine number and distribution	in medulla	present in subcortical region	numerous in medulla	numerous in the medulla	numerous in medulla	few in medulla
Appearance of medulla in cross-section	rounded cells	rounded cells	rounded cells	rounded cells	rounded cells	rounded cells
Apical cell character	pronounced	pronounced	sunken in emarginate apex or apical groove	pronounced	pronounced	pronounced
Tetrasporangial sori	unknown	borne distally in rounded sori	borne terminally; ill-defined in small thalli (<1 cm), well-defined in large thalli.	borne in distal marginal bladelets	borne in stichidium-like enlargements near base of plant	borne terminally, tetrasporangia in V-shaped rows
Bisporangial sori	unknown	unknown	not found	not found	not found	not found
Cystocarps	unknown	unknown	Bilocular, borne distally	unilocular, borne subterminally	unilocular, intercalary to subterminally positioned	unilocular

CHAPTER 7

BIOGEOGRAPHY OF SOUTH AFRICAN GELIDIACEAE (RHODOPHYTA)

INTRODUCTION

A number of South Africa herbaria house important algal collections. Richard Norris compiled the largest collection of South African Gelidiaceae, housed at the Botany Department Herbarium of the University of Natal, Pietermaritzburg (NU) (pressed specimen collection) and at the Department of Animal, Plant and Environmental Sciences of the University of the Witwatersrand (wet collection). The collection focuses mostly on the seaweeds of the KwaZulu-Natal Province (KZN) coast (Fig. 1) but also includes specimens from the South African Museum collection formerly housed in Kirstenbosch, making it quite representative of South African Gelidiaceae as a whole. The Albany Museum in Grahamstown (GRA) houses the very extensive collection of Mary A. Pocock focusing mostly on seaweeds of the Eastern Cape coast. The Bolus Herbarium at the Botany Department of the University of Cape Town (BOL) houses another important algal collection focused mostly on specimens from the Cape region and also contains my South African east coast collection. Almost all the specimens in these collections provide information on the collection locality and some provide information on the ecology (including vertical distribution) of the specimen. These collections are the most valuable sources of biogeographic information of South African Gelidiaceae due to the number of specimens they house collected from local shores. I estimate that there are in excess of 3000 specimens of Gelidiaceae countrywide in these collections. A number of species checklists have been published for various locations along the South African coast (Anderson and Stegenga 1989, Drège 1843, Bolton and Stegenga 1987, 1990, 2002, Farrell *et al.* 1993, Jackelman *et al.* 1991, Seagrief 1980). These, together with a few original species descriptions, reviews and reports of new records for South Africa (Anderson 1994, Anderson and Bolton 1985; Dickinson 1950; Millar and Freshwater 2004; Norris 1990b; Norris, Hommersand & Fredericq 1987; Stegenga *et al.* 1997), provide valuable biogeographic information. Only two publications to date synthesise biogeographic information on South African Gelidiaceae: Norris (1992b) on the distribution of the species occurring in KZN, and Stegenga *et al.* (1998) on the distribution of the species occurring along the South African West Coast. The aim of this chapter is to provide a detailed synthesis of horizontal and vertical distribution information taken from these various sources for species in the Gelidiaceae occurring along the entire length of the South African coast.

MATERIALS AND METHODS

Specimens were collected from the intertidal and shallow subtidal of inshore reefs as well as from offshore reefs using SCUBA at various locations along the South Africa coastline from 1999 to 2002. Specimens were pressed, dried in silica gel or preserved in 5% Formalin in seawater, subsequently identified and their collection location and vertical distribution recorded. Various national herbaria were visited (BOL, GRA, NU and the Norris wet-collection at WITS University) that house all the major South African seaweed collections, as well as some international herbaria housing South African specimens (GENT, LD, S). The specimens for which identifications could be confirmed or corrected were documented, including their collection location and any ecological information provided on the label. In the case of multiple specimens of a species collected from one location, at least one specimen was documented, the rest being ignored. Specimens collected from drift were also ignored except in the case of rarely encountered species or those only known from drift collections. Distributional data was also gathered for the species of *Gelidiella* Feldmann and Hamel occurring in South Africa.

RESULTS

More than 500 specimens (Appendix 2) in total were documented and used to compile the horizontal distributions (Table 7.1) of all currently recognized species of South African Gelidiaceae as well as three species of *Gelidiella* and three undescribed species considered in the previous chapter, i.e. *Gelidium* sp., *Pterocladiella* sp. and *P. 'caerulescens'*. The horizontal species distributions were determined by subdivided the South African coastline into 58 x 50 km sections (Table 7.2), and scoring the sections for the presence of a species if a specimen of that species had been collected from a location

sections represented by location code. See Table 7.2 for key to location code.
cimen

36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				■		■
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Table 7.1. Distribution of Gelidiales species (and three Gelidiella species) in South African. South African coastline subdivided into

■ = record from specimen collection locations ▨ = record from literature ▩ = record from drift s

Location Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Gelidium abbottiorum		■																	■			■	■	■		■		■	■	■	■			■
G. applanatum																■		▨																
G. arenarium																		■					■					■	■		■	■	■	■
G. capense												■			■	■	■	■	■	■		■	■	■				■				■		
G. crinale																	■																	
G. declerckianum																																		
G. foliaceum																																		
G. isabelae																		■																
G. micropterum			■									■			■	■	■	■																
G. minusculum																																		
G. pteridifolium															■			■	■			■		■										
G. profundum																																		
G. pristoides															■	■	■	■	■	■		■	■	■					■	■	■	■		■
G. reptans																		▨	▨			■	■											
G. vittatum		■			■			■	■					■	■	■	■	■	■	■	■	■		▩							▩			
Gelidium sp.																																		
Pterocladiella bartlettii																																		
P. 'caerulescens'																																		
P. caespitosa																																		
P. caloglossoides																		■																
Pterocladiella sp.																																		
Ptilophora coppejansii																																		
P. diversifolia																																		
P. helenae																																		
P. hildebrandtii																																		
P. leliaertii																																		
P. pinnatifida																																		
P. rhodoptera																																		
P. rumpii																																		
Gelidiella acerosa																																		
G. antipae																		■																
G. lubrica																																		

Table 7.2. Key to location code in Table 7.1. Location codes refer to 58 x 50 km sections of the South African coastline. Also listed are some localities occurring within the coastal sections (adapted from Table I in Bolton and Stegenga, 2002). Pt. = Point.

Code	Limits of coastal sections	Localities occurring within the coastal sections
1	Orange River to Holgats River	
2	To just south of Wedge Pt.	Peacock's Bay, Port Nolloth, McDougall's Bay
3	To just south of Melkbos Pt.	Kleinzee, Buffels River
4	To Swartlintjies River	Skulpfontein Pt., Swartlintjies River
5	To 10 km north of Groen River	Cape Hondeklipbaai. Spoeg River
6	To just north of Brak River	Groen River, Island Pt., Blougat
7	To just north of Duiwegat	Voelklip, Sout River, Blinkwater Bay
8	To just south of Doring Bay	Olifants River, Strandfontein, Doring Bay
9	To just north of Elands Bay	Lambertsbaai, Lang River
10	To just north of Laaiplek	Elands Bay, Die Vlei, Dwarskersbos
11	To just south of Cape Columbine	Laaiplek, St. Helena Bay, Paternoster
12	To just east of Saldanha	
13	To Postberg	Langebaan Lagoon Saldanha Bay
14	To just south of Modde River	Yzerfontein, DassenIs., Grotto Bay
15	To Sea Pt.	Melkbosstrand, Table Bay, Mouille Pt., 3-Anchor Bay
16	To just north of Scarborough	Sea Pt., Camps Bay, Bakoven, Oudekraal, Oude Schip, Vulcan Rock, Kommetjie, Slangkop Pt.
17	To just east of Kalk Bay	Olifantsbosch, Platboombaai, Buffelsbaai, Miller's Pt., Oatlands Pt., Glencairn, Clovelly, Kalk Bay
18	To just south of Gordons Bay	Dalebrook, St. James, Muizenberg, Strandfontein, Swartklip, Eersterivier, Strand, Gordons Bay
19	To just east of Kleinmond	Rooi Els, Pringle Bay, Hangklip, Betty's Bay
20	To just south of Danger Pt.	Sandbaai, Hermanus, Voelklip, Stanford Beach (Gansbaai), Franskraal
21	To just east of Quoin Pt.	Danger Pt., Pearly Beach , Dyer Islands
22	To just east of Struis Bay	Die Mond, Brandfontein, Cape Agulhas
23	To just east of Skipskop	Struis Bay, Arniston
24	To just east of Cape Infanta	Koppie Alleen, Cape Infanta, Bree River, Witsands, DeHoop Nature Reserve
25	To just east of Grootjongensfontein	Puntjie, Skurwe Bay
26	To just west of Gouritzmond	Jongesfontein, Stilbaai (Preekstoel), Bloukrans, Bull Pt.
27	To just north of Mossel Bay	Gouritzmond, Vlees Bay, Pinnacle Rock
28	To just west of Victoria Bay	Hartenbos, Tergniet, Great Brak rivers, Herolds Bay
29	To just west of The Heads, Knysna	Victoria Bay, Wilderness, Sedgefield, Buffalo bay, Walker's Bay
30	To Jack's Pt.	The Heads, Knysna (Blauwklip), Neusgat
31	To Elandbos River	Plettenberg Bay, Nature's Valley, Arch, Die Punt, Blousloep
32	To Skuinsklip	Storms River, Voelkrans, Skietgate, Groot Rivier
33	To Thys Pt.	Aasvoelklip, Titsikama River, Klipdrif River
34	To just west of Gamtoos River	Cape St. Francis, Krom River, seekoei River, Jeffreys Bay
35	To just east of Sardinia Bay	Van Staadens River, Claasen Pt.
36	To just east of St. George's Beach	Chelsea Pt., Sea View, Port Elizabeth, Algoa Bay, Bluewater Bay
37	To just west of Woody Cape	St. Croix Is., Sundays River, Bird Island
38	To just west of Kenton-on-Sea	Seal Is., Bird Is., Cape Padrone, Cannon Rocks, Boknes
39	To just east of Kleinemonde	Bushmans River, Kenton on Sea, Kasouga, Port Alfred/Kowie, Riet Rivier
40	To just east of Keiskamma River	Kleinemonde, Fish Pt., Hamburg, Waterloo Bay,
41	To just east of East London	Chalumna, Kidds Beach, Nahoon Reef, Cove Rock
42	To Haga-Haga	Gonubie, Glengariff, Haga Haga,
43	To Qora River	Double Mouth, Cape Morgan, Kei Mouth, Qolora, Qora, Centani Coast, Mazeppa Bay
44	To just east of Xora River	Dwessa, The Haven, Bashee R. mouth, Breezy Pt.,
45	To Shark's Pt.	Presley's Bay, Coffee Bay, Hluleka
46	To Mkozi River	Port St. Johns, UmTafuf, Dome Bluff, Mbotyi, Manteku
47	To Mnyameni River	Cathedral Rock, Lambasi Bay, Gweqwe (Mkambati), Wild Coast
48	To just north of St. Michaels-on-Sea	Mzamba, Port Edward, Glenmore, Palm Beach, Trafalgar, Marina Beach, Southbroom, Uvongo, St. Michaels-on-Sea
49	To just south of Pennington	Protea Banks, Shelly Beach, Port Shepston, Hibberdene, Umtentweni, Ifafa Beach
50	To just south of Isipingo Beach	Pennington, Umdoni Park, Park Rynie, Widenham/Umkomas, Doonside, Tiger Rocks
51	To Desainagar	Isipingo, Reunion Rocks, Treasure Beach, Bluff, Umhlanga Rocks, Umdloti
52	To just north of Zinkwazi Beach	Tongaat Beach, Ballito, Shaka's Rock, Sheffield Beach, Tinley Manor, Mvoti
53	To just east of Mtunzini	Tugela River, Dunn's Reserve
54	To Mbonambi Beach	Richards Bay
55	To just north of First Rock	Dawson's Rocks, Cape St. Lucia, St. Lucia, First Rocks
56	To Bhukwini	Perrier's Rocks, Mission Rocks, Cape Vidal, Leven Pt.
57	To just north of Gobey's Pt.	Liefeldts Rocks, Sodwana Bay, Leadsman Shoal
58	To Kosi Bay	Mabibi (Hulley Pt.), Lala Nek, Black Rock, Boteler Pt., Tiger Reef, Kosi Bay

Table 7.3. Vertical distribution of South African Gelidiaceae taxa (and three Gelidiella species). Records from intertidal/subtidal fridge have been included in shallow subtidal column. Known distribution (black); expected distribution (grey).

	Upper intertidal	Mid intertidal	Lower intertidal	Subtidal (<-10 m)	Subtidal (>-10 m)
Gelidium abbottiorum		■	■	■	
G. applanatum				■	■
G. arenarium		■			
G. capense			■	■	
G. crinale			■	■	
G. declerckianum	■	■	■		
G. foliaceum	■	■			
G. isabelae	■	■	■		
G. micropterum		■	■	■	
G. pteridifolium			■	■	
G. profundum					■
G. pristoides	■	■	■		
G. reptans	■	■	■	■	
G. vittatum			■	■	
Gelidium sp.				■	
Pterocladiella bartlettii				■	
P. 'caerulescens'				■	
P. caespitosa			■		
P. caloglossoides				■	■
Pterocladiella sp.				■	
Ptilophora coppejansii					■
P. diversifolia					■
P. helenae				■	▨
P. hildebrandtii					■
P. leliaertii					■
P. pinnatifida				■	■
P. rhodoptera					■
P. rumpii				▨	▨
Gelidiella acerosa		■	■	■	
G. antipae				■	■
G. lubrica		■	■		

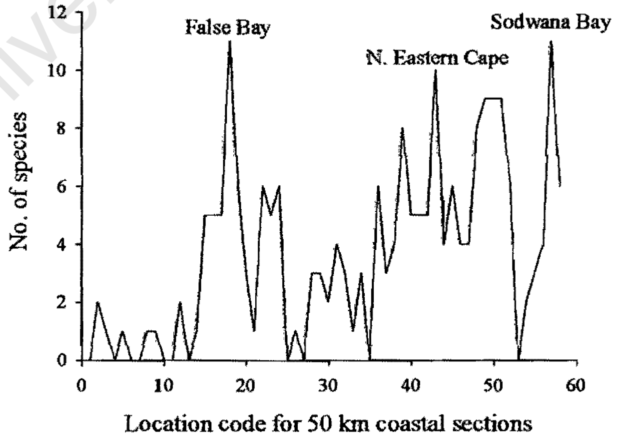

Fig. 7.1. Number of species of South African Gelidiaceae (including three *Gelidiella* species) per 50 km section of the South African coast.

within a section. The number of species per 50 km section was also plotted (Fig. 7.1). The vertical species distributions (Table 7.3) were compiled from specimen labels, literature and personal observations.

DISCUSSION

The distributional data are incomplete since algal collections have been concentrated in easily accessible areas, mostly around the Cape Peninsula, Cape Agulhas region, major towns along the central to northern Eastern Cape coast and the entire length of the KZN coast (Table 7.1). Since taxonomic research has also been focused predominantly on the Gelidiaceae of these areas, they tend to have the highest number of species recorded as well (Fig. 7.1). In addition, the distributions of some species are poorly known due to collector bias. *Gelidium abbottiorum* R.E. Norris, *G. capense* (Gmelin) Silva, *G. pteridifolium* Norris, *G. pristoides* (Turner) Kützing, and *G. vittatum* (Linnaeus) Kützing are large, frondose species that are easily identifiable because of their size and are relatively easy to collect given their occurrence in the intertidal and shallow subtidal, and their distributions are quite well known as a consequence. However, some of the remaining species such as those of *Ptilophora* Kützing are relatively inaccessible due to their occurrence in deep subtidal habitats, whilst other species have a turf habit and are generally overlooked because of their small size and the difficulty involved in their identification. Numerous specimens of turf species are housed in the various South African herbaria and are either incorrectly identified or identified to the genus level only and filed as *'species incertae'*. Efforts were made to identify and document these specimens but there still remains much scope for research on turf species in South Africa.

The species of *Gelidium* Lamouroux tend to be widespread in southern Africa, four being distributed from tropical East Africa to the South African west coast. About half these species appear to have affinities with the South African south coast (Agulhas) and/or west coast floristic regions (*sensu* Bolton *et al.* 2003). Species of *Ptilophora, Pterocladiella* Santelices and Hommersand and *Gelidiella* are predominantly found along the East African coast in both tropical and temperate waters, some species found along the south coast to as far west as False Bay. *Gelidium profundum* Tronchin, *Ptilophora leliaertii* Tronchin, *P. coppejansii* Tronchin, and *P. rhodoptera* Norris appear to have relatively restricted distributions, occurring only along the region of overlap between the South African south coast and tropical Indo-Pacific floristic regions (*sensu* Bolton *et al.*, 2003) situated between Hluleka (EC) and St Lucia (KZN) and are endemic to this region. *Gelidium minusculum* (Weber-van Bosse) R.E. Norris has similarly been found in this region, at Mvoti in northern KZN, but was originally described from Indonesia and subsequently found in the Mediterranean and along the coast of Queensland (Australia) and Brazil (Norris, 1992b). The identity of this species from South African specimens is in question. *Ptilophora diversifolia* (Suhr) Papenfuss, *P. pinnatifida* J. Agardh, *P. helenae* (Dickinson) Fan & Papenfuss and *Gelidium foliaceum* (Okamura) Tronchin appear to have affinities for this region of overlap, with distributions that extend slightly into the tropical east coast and/or south coast floristic regions as well.

Species which appear to have tropical East African affinities include *Gelidium reptans* (Suhr) Kylin and *G. arenarium* Kylin, found in False Bay and spreading eastwards into tropical East Africa, with *G. reptans* reported from Mozambique (Isaac 1956, Critchley *et al*. 1997, Silva *et al.* 1996) and *G. arenarium* from Kenya (Isaac 1971, Lawson 1980, Silva *et al.* 1996). The South African distribution of *Gelidiella acerosa* (Forsskål) J. Feldmann and G. Hamel is well known, occurring in the northern KZN, northern Mozambique (Bandeira *et al.*, 2001), Djibouti, Kenya, Tanzania (Jaasund 1976, Lawson 1980) and Somalia (Silva *et al.*, 1996). *Pterocladiella caespitosa* (Howe) Santelices is widespread in KZN and reported as far west as Hluleka (Bolton and Stegenga, 1987) in the Eastern Cape. The species has an East African distribution according to Farghaly (1980), reported from Mozambique (Critchley *et al.* 1997, Felicinin and Perrone 1994, Isaac 1956, Silva *et al.* 1996) and Kenya (Lawson 1980, Isaac 1971, Silva *et al.* 1996). *Ptilophora hildebrandtii* (Hauck) Norris and *P. rumpii* (Dickinson) Norris are known from northern KZN and tropical East Africa as far as Kenya (Chapter 5). *Gelidiella lubrica* (Kützing) Feldmann *et* Hamel, *Pterocladiella bartlettii* Taylor, *Gelidium sp.*, *Pterocladiella 'caerulescens'* and *Pterocladiella* sp. are only known from the northern half of KZN, particularly from localities north of St Lucia, and hence are likely to have tropical East African affinities.

Various species appear to have a south coast affinity. *Gelidium abbottiorum* and *G. pteridifolium* are widely distributed along the south and east coasts. *Gelidium abbottiorum* occurs to just west of Cape Agulhas, being absent along the entire west coast except for a record from MacDougal's Bay near the Namibian border. *Gelidium pteridifolium* has been reported along the west coast at Muizenberg and in Table Bay and Saldanha Bay, but is believed to be distributed only as far west as Muizenburg. The Saldanha Bay record (Schils, 1998) was a misidentification of *G. capense* and the Table Bay record is believed to be the result of an historical mislabelling. Tyson collected a number of specimens of *G. pteridifolium* in Table Bay around the turn of the 20th century, but the species has not been found there since, despite extensive collecting in this area in recent years. Thus, the collection location of these specimens is believed to be inaccurate as stated in Norris, Hommersand and Fredericq (1987). *Gelidium capense* is found along the Western Cape coast, and there are scattered reports of it extending up the east coast to southern KZN. Thalli of this species occurring in False Bay tend to be loosely branched and may lose their distinctive geniculate branch bends. Various researchers have misidentified these thalli as those of *G. pteridifolium*. *Gelidium pristoides* has a similar distribution to that of *G. capense* (Table Bay to southern KZN). *Gelidium isabelae* Taylor is a poorly known species in South Africa having been reported only from False Bay and Port Elizabeth. *Gelidium declerckianum* Tronchin is a newly described species and is thus far only known from Kidd's Beach and East London in the Eastern Cape.

Species with an entirely west coast distribution include *Gelidium vittatum* and *G. applanatum* Stegenga, Bolton *et* Anderson. *Gelidium vittatum* is found along the Western and Northern Cape coast extending into Namibia. Cape Agulhas is believed to be the easternmost limit of its distribution according to Anderson (1994) and Anderson and Bolton (1985). Three *G. vittatum* specimens collected east of Cape Agulhas exist in GRA and NU. These were collected from Witsands (near the Brede River mouth), Natures Valley and Port Elizabeth, being roughly 100, 450 and 600 km east of Cape Agulhas respectively. It is though that these specimens were more than likely epiphytes collected from drift *Ecklonia maxima* (Papenfuss) Osbeck. *Gelidium applanatum* has only been found at two localities along the Cape Peninsula, not surprising considering the minute size of its thallus.

The floral affinity of some species is uncertain due to collections being too few and/or too scattered. *Pterocladiella caloglossoides* (Howe) Santelices has been recorded from False Bay and central KZN, *Gelidiella antipae* Celan from False Bay and northern KZN, and *Gelidium crinale* (Turner) Gaillon from False Bay, the south coast and east coast. *Gelidium crinale* has also been reported from Kenya (Isaac, 1967) and tropical West Africa (Lawson and John, 1987), though apart from the False Bay collection, it is apparently absent from the South African west coast. *Gelidium micropterum* Kützing occurs on the west coast of South Africa, known predominantly from the Cape Peninsula and False Bay, but has been recorded from the east coast and Tanzania (Jaasund, 1976) as well.

A number of *Gelidium* species have relatively broad vertical distributions, some distributed from the mid-intertidal to subtidal, whilst others span the entire intertidal range (Table 7.3). Species of *Pterocladiella* appear more restricted in their vertical distribution and tend to occur subtidally (including tidal pools). *Gelidium applanatum*, *G. profundum*, *Pterocladiella caloglossoides*, *Gelidiella antipae* and species of *Ptilophora* are found in the subtidal and extend to depths of more than -10 m. Details on the vertical distribution of each species are as follows:

Gelidium abbottiorum: found on vertical or flat rock surfaces, on coralline crusts and barnacles, in pools and sandy gullies, from mid-intertidal to subtidal.

Gelidium applanatum: subtidal, epizoic on *Pyura* spp. at -15 m, as well as epiphytic on a piece of drift *Ecklonia maxima*.

Gelidium arenarium: forming upper-intertidal turf in sandy habitats.

Gelidium capense: on rock and abalone from the subtidal fringe down.

GENERAL DISCUSSION

By subsuming *Onikusa* and *Suhria* into *Gelidium* and supporting the merger of *Beckerella* and *Ptilophora* (Norris, 1987), the total number of genera in the Gelidiaceae occurring in South Africa has been reduced to three, though analyses of molecular data have indicated that there may be a fourth, undescribed genus occurring along the northern east coast. A practicable phylogeny-based classification of the South African Gelidiaceae has resulted. Future research should be aimed at evaluating the taxonomic status of *Capreolia* and *Acanthopeltis* and restoring monophyly to *Gelidium* by recognising the *rbc*L species clades, containing these two genera (Freshwater *et al.* 1995, Shimada *et al.* 1999) and other *Gelidium* species, at the genus level. This can be followed by the determination of morphological synapomorphies for the remaining *rbc*L species clades containing *Gelidium* species.

This study provides the first comprehensive review of *Ptilophora*. The region of highest *Ptilophora* species diversity in the world was found to be the KwaZulu-Natal (KZN) coast (Fig. 2). Protea Banks Reef (southern KZN) has particular conservation importance with respect to *Ptilophora* since five of the eight South African *Ptilophora* species occur on this relatively short reef (~10 km long). The high incidence of sponge associations in *Ptilophora* species may make this genus of particular interest in the expanding field of research on sponge-algal interactions. The accessibility of Protea Banks by numerous dive-charter companies would facilitate research of this kind on *Ptilophora*.

This study also provides the first comprehensive review of the diversity and distribution of the Gelidiaceae in South Africa and increases the species diversity in this family by 40%, by the addition of one new record (*Pterocladiella bartlettii*), four new species (*Ptilophora coppejansii*, *P. leliaertii*, *Gelidium declerckianum* and *G. profundum*) and three uncertain species that fit a broad morphological description of *Pterocladiella caerulescens*. There are now 29 species in total, 16 of which belong to *Gelidium*, eight belong to *Ptilophora* and five belong to *Pterocladiella*.

The South African distributions of eleven Gelidiaceae species are extended here (Fig. 2 – it should be borne in mind that some species distributions are inferred from relatively few

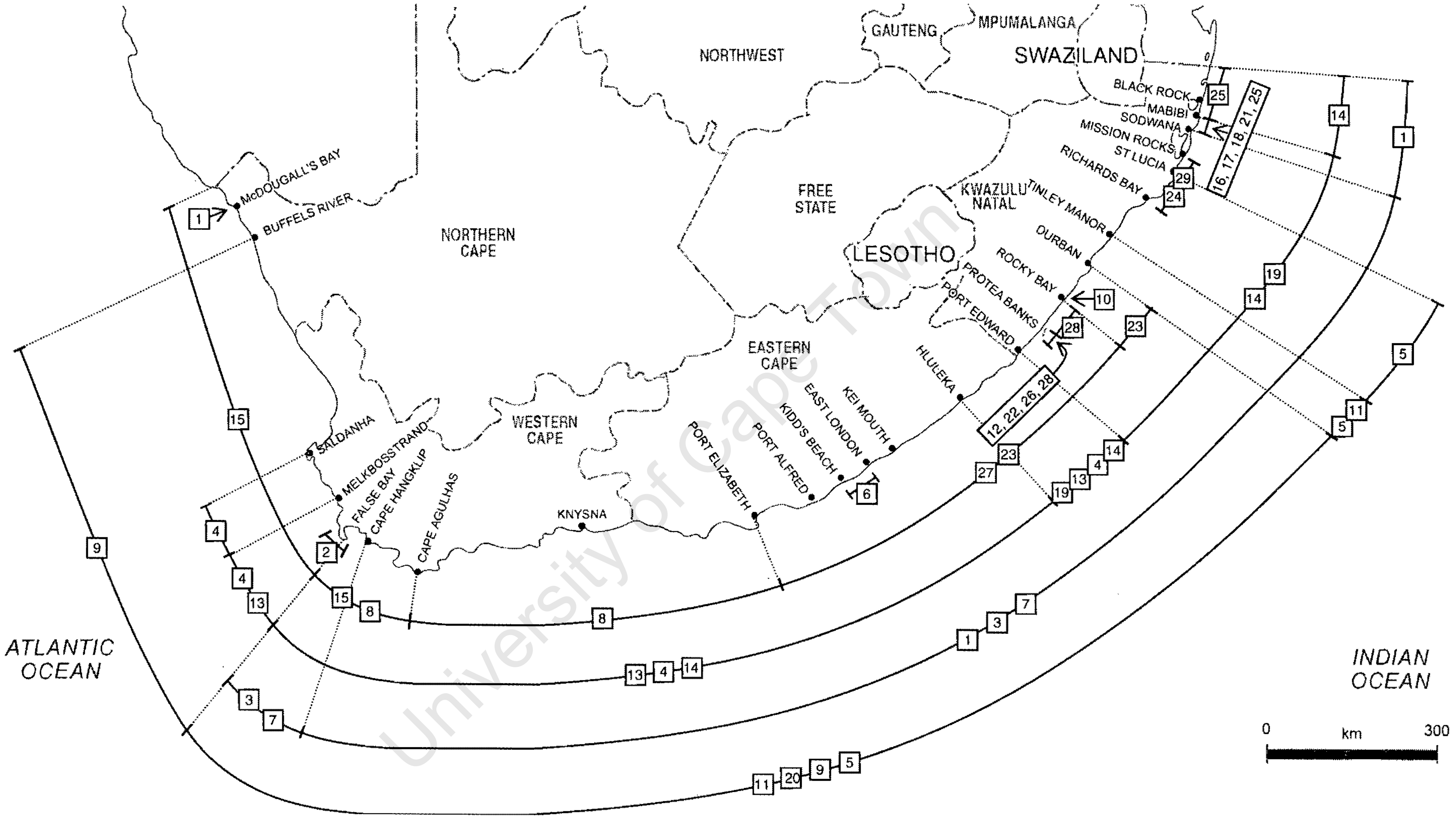

Fig. 2. Distributions of Gelidiaceae species in South Africa. Sodwana refers to the coast between Jesser Point and 9-Mile Reef. *Gelidium abbottiorum* (1), *G. applanatum* (2), *G. arenarium* (3), *G. capense* (4), *G. crinale* (5), *G. declerckianum* (6), *G. foliaceum* (7), *G. isabelae* (8), *G. micropterum* (9), *G. minusculum* (10), *G. pteridifolium* (11), *G. profundum* (12), *G. pristoides* (13), *G. reptans* (14), *G. vittatum* (15), *Gelidium* sp. (16), *Pterocladiella bartlettii* (17), *P. 'caerulescens'* (18), *P. caespitosa* (19), *P. caloglossoides* (20), *Pterocladiella* sp. (21), *Ptilophora coppejansii* (22), *P. diversifolia* (23), *P. helenae* (24), *P. hildebrandtii* (25), *P. leliaertii* (26), *P. pinnatifida* (27), *P. rhodoptera* (28), *P. rumpii* (29).

and scattered reports, see chapter 7). *Gelidium abbottiorum* was previously known to occur on the east coast to as far west as False Bay, but is now reported from McDougall's Bay, just south of Port Nolloth. This species is otherwise absent along the South African west coast, making this report seem questionable. However, a few Eastern Cape species such as *Plocamium beckeri* Simons that do not occur along the west coast of South Africa, reappear in Namibia, Angola (Hommersand, 1986) and McDougall's Bay (Bolton 2002: unpubl. data), suggesting that formerly there may have been a continuous distribution of these algae (Hommersand, 1986). *Gelidium abbottiorum* has been collected as far east as Kosi Bay just south of the Mozambican border and probably extends across the border. *Gelidium arenarium* was previously only known from KZN: from the type locality, Isipingo, near Durban (Kylin 1956) and specimens grown in culture originally collected from a reef at Mvoti River Mouth, north of Durban (Norris 1992). This study extends the northern limit of its distribution to Jesser Point (Sodwana) and southern limit to False Bay. *Gelidium crinale* had only been reported from Cape Morgan (Delf and Michell, 1921), but is now known to occur from False Bay to St. Lucia. *Gelidium isabelae*, a newly reported species from False Bay, has also been found at Port Elizabeth. *Gelidium micropterum* was reported to inhabit the cold west coast waters of South Africa (Papenfuss, 1952), in particular the coast around the Cape Peninsula to Cape Hangklip, and to occur along the south coast as far east as Knysna (Stegenga *et. al.*, 1997). In this study a specimen was located from Buffels River (northern west coast), in accordance with Papenfuss (1952), and from Paternoster near Saldanha. The east coast distribution of this species has been extended to central KZN. The west coast distribution of *G. pristoides* has been slightly extended from Sea Point, according to Stegenga *et al.* (1997), to Melkbosstrand. The distribution of *G. pteridifolium* is similarly slightly extended. Norris (1992) stated that it occurred along the southern KZN coast to as far as Durban. It's range is now extended to Tinley Manor (KZN North Coast). *Pterocladiella caloglossoides* and *P. caespitosa* had been reported from central KZN. The range of *P. caloglossoides* is now known to extend to False Bay, and the range of *P. caespitosa* now extends from Hluleka (northern Eastern Cape) to Mabibi (northern KZN). *Ptilophora hildebrandtii* had only been reported from 5-Mile Reef at Sodwana but was recently collected from Tiger Reef, extending its distribution to the Mozambican border. Norris described *Ptilophora rhodoptera* from Rocky Bay, KZN. Its range is now known to extend southwards to Protea Banks (southern KZN).

Ptilophora helenae was found attached for the first time in this study, having been previously reported from drift collections only. Epilithic specimens were collected from Mission rocks, just north of St. Lucia. The flora of the coastline between St. Lucia and Sodwana is a gap in our knowledge since collections from this stretch of coast have been few and mostly from the intertidal and shallow subtidal. This region has consequently become a focal point for current systematic research by South African and Belgian researchers. New collections from this region should greatly improve our knowledge of its flora and it is hoped that they will determine the location of epilithic populations of *Ptilophora rumpii*, a species only known from drift collections to date but expected to occur in this region.

Norris (1992) provides the only report of the distribution of *Gelidium foliaceum*, stating that the species occurs along the east and south coast from KZN to Cape Town, listing various specimens collected in Natal. In this study, specimens of this species were distributed along the east coast from Sodwana to Kei Mouth. It is possible that the south coast distribution of *G. foliaceum* reported by Norris refers to the *G. pristoides* turf-form ecotype identified in chapter 1 since these two species are virtually identical in gross morphology. However, the distribution proposed by Norris is maintained for the time being.

By the time Richard Norris concluded his research on South African Gelidiaceae at the beginning of the 1990s, the region of highest diversity in South Africa for the Gelidiaceae was the east coast, particularly the northern Eastern Cape and KZN coast: five species had been reported to occur on the west coast (from the Namibian border to Cape Agulhas), six on the south coast (from Cape Agulhas to Port Elizabeth), and fifteen on the east coast (from Port Elizabeth to the Mozambican border). It is now known that eight species occur on the west coast, ten on the south coast and 26 on the east coast of which 25 occur along the KZN coast. The high species diversity of the KZN coast is most probably related to the fact that it is a region of overlap between two floristic regions: the temperate South African south coast and tropical Indo-Pacific floristic regions (Bolton *et. al.*, 2003).

The diversity of South African Gelidiaceae (29 species, 3 genera) is comparable to the well known Gelidiaceae of Japan which has at least 22 species in four genera (Shimada *et al.* 2000a & b, Yoshida 1998). South Africa appears to be species rich when compared to other regions where the diversity of the Gelidiaceae is believed to be well known, such as Chile which has 3 *Gelidium* species (Hoffmann and Santelices 1997, Santelices and Abbott 1985, Santelices and Montalva 1983) and southern Australia which has 7 species in 4 genera (Womersley 1994, Guiry and Womersley 1993). A possible reason for the considerably higher diversity may lie in the range of habitats along the South African coast, ranging from the cold temperate waters of the west coast to the warm temperate and tropical waters of the east coast.

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APPENDIX 1

GLOSSARY

Acropetal: Developing in a succession from the base towards the apex

Acuminate: Tapering gradually from the base to a sharp point

Acute: Terminating with a sharp or well defined angle

Alate: Having a midrib beset on either side by flattened wings

Anticlinal: Referring to cells oriented perpendicularly to the thallus surface

Bipinnate: Twice pinnate

Caespitose: Growing in tufts; forming mats or tufts; often refers to a short plant with many stems or branches, forming a cushion appearance

Cordate: Having a heart-shaped outline

Crenate: Dentate with the teeth much rounded

Cuneate: Triangular with the acute angle downwards

Distal: The upper part of the thallus

Decurrent: Directed towards the base of the plant, i.e. branching angle is 90-180°

Determinate: Referring to a branch with limited growth with respect to growth that may be attained by other branches of the same order.

Distichous: Arranged in two vertical rows on opposite sides of an axis

Emarginate: Having a shallow notch at the tip

Filiform: Thread-shaped; long, slender and terete

Hapteron: Holdfast; brush-type aggregation of anchoring, aseptate cells produced on rhizoidal branches

Indeterminate: Referring to a branch of apparently unlimited growth

Intercalary: Inserted equidistant from the apex and the base (could originally be inserted terminally but ceasing to be so when vegetative growth subsequently resumes from the apex)

Lanceolate: Shaped like a lance-head, several times longer than wide, broadest above the base and tapering gradually to the apex.

Ligulate: Strap-shaped

Mucronate: Terminated with a rounded or obtuse apex bearing an abrupt, narrow protuberance/papilla

Oblanceolate: Tapering gradually basally, with the broadest part towards the apex

Obtuse: tapering slightly to a rounded end.

Papilla: A small nipple-like projection

Pectinate: Referring to branching that is comb-like

Periclinal: Referring to cells oriented parallel to the thallus surface

Pinnae: The primary divisions of a pinnate or compoundly pinnate frond

Pinnatifid: Divided in a pinnate manner where alae of pinnae merge into alae of bearing branch

Pinnate: Having a central axis bearing a bilateral series of shorter, often determinate branches, arranged oppositely to alternately

Pinnule: An ultimate branch of a pinnately branched frond

Procurrent: Referring to a branch that is directed distally, i.e. branching angle is 0-90°

Protuberant: Prominent, bulging beyond the adjacent surface

Proximal: Referring to the lower part of the thallus

Quadripinnate: Four times pinnate

Quercifoliate: shaped like an oak leaf

Ramuli: branchlets or secondary branches

Retuse: With a shallow notch at a rounded apex

Rostrate: Shaped like a long, curved beak

Subulate: Tapering upward from a triangular base to a slender point

Surface proliferations: A short, determinate branch produced adventitiously on the surface of a flattened blade

Tridentate: Bearing three adjacent, sharp teeth that are usually equally prominent

Tripartite: Referring to an apex terminating in three parts equal in prominence

Tripinnate: Three times pinnate

APPENDIX 2

Table 1. Specimens and literature used to compile distribution information of South African Gelidiales species. Specimens are listed in geographical order from east to west along the South Africa coast. At least one specimen or literature reference is included for every collection location, some locations may have multiple specimens. KwaZulu-Natal (KZN), Eastern Cape (EC), Transkei (TSK), Ciskei (CSK), Western Cape (WC), Northern Cape (NC), Bolus (BOL), Natal University (NU), Albany Museum (GRA), Norris wet collection at WITS (WITS).

Prov.	Locality	Specimen identification	Specimen number(s)	Determinavit/ collector	Herbarium
Gelidiella acerosa					
KZN	Boteler Pt.	Gelidiella acerosa	SAM100443/ NAT5562	Balkwill	NU
KZN	Black Rock	Gelidiella acerosa	100442 / NAT5220	Olivieri	NU
KZN	Lala Nek	Gelidiella acerosa	NAT5638	Phelan	NU
KZN	Sodwana (9-Mile reef)	Gelidiella acerosa	KZN522B	Tronchin	BOL/GENT
KZN	Sodwana - Jesser Point	Gelidiella acerosa	SAM102038/ NAT6265	Norris	NU
KZN	Sodwana Bay	Gelidiella acerosa	SAM102038/ NAT6265	Norris	NU
KZN	Sodwana	Gelidiella acerosa	KZN456B	Tronchin	BOL/GENT
Gelidiella antipae					
KZN	Leadsman Shoal, St. Lucia	Gelidiella antipai	NAT2392	Bouwer	WITS
WC	St. James	Gelidiella antipai	Sa88	Stegenga	BOL
Gelidiella lubrica					
KZN	Sodwana	Gelidiella lubrica	SAM102153/ NAT6265	Norris	NU
KZN	Perrier's Rocks	Gelidiella	2155	Pocock	GRA
KZN	Treasure Beach	Gelidium caespitosum	KZN122	Tronchin	BOL/GENT
KZN	Rocky Bay - Park Rynie	Gelidiella lubrica	SAM100452/ NAT2107	R. Pienaar	NU
KZN	Rocky Bay	Gelidiella pannosa/lubrica	009387 / NAT2107	R. Pienaar	NU
Gelidium abbottiorum					
KZN	Kosi Bay	Gelidium amansii	NAT720	Norris	WITS
KZN	Black Rock	Gelidium abbottiorum	910100 / NAT3768	Norris	NU
KZN	St. Lucia - Cape Vidal	Gelidium abbottiorum	009047 / NAT2224	Norris	NU
KZN	Mission Rocks	Gelidium abbottiorum	3131	Norris	NU
KZN	Perrier's Rocks	Gelidium amansii	9748	M. A. Pocock	GRA
KZN	Umvoti Mouth	Gelidium abbottiorum	008102 / NAT1669	Norris	NU
KZN	Tinley Manor	Gelidium abbottiorum	SAM100292/ NAT6095	Norris	NU
KZN	Sheffield Beach	Gelidium abbottiorum	NAT6103	Clapham	WITS
KZN	Shaka's Rock	Gelidium amansii	2578	Simons	BOL
KZN	Shaka's Rock	Gelidium abbottiorum	Sp26	Tronchin	BOL
KZN	Ballito Bay	Gelidium abbottiorum	010129 / NAT4431	Norris	NU
KZN	Tongaat Beach	Gelidium amansii	8518	Norris	NU
KZN	Umdloti - Sentinel Rocks	Gelidium abbottiorum	sn1241	Simons	BOL
KZN	Umdloti - Sentinel Rocks	Gelidium abbottiorum	007042 / NAT1055	Norris	NU
KZN	Treasure Beach	Gelidium abbottiorum	KZN69	Bolton et al.	BOL/GENT
KZN	Reunion Rocks	Gelidium amansii	9952	M. A. Pocock	GRA
KZN	Reunion Rocks	Gelidium abbottiorum	SAM100328/ NAT1795	Norris	NU
KZN	Isipingo	Gelidium amansii	9915	M. A. Pocock	GRA
KZN	Tiger Rocks	Gelidium abbottiorum	SAM100224/ NAT6153	J. Junor	NU
KZN	Tiger Rocks	Gelidium abbottiorum	SAM100250/ NAT6144	F. Junor	NU
KZN	Doonside	Gelidium amansii	9707	Norris	NU
KZN	Umkomas/Widenham	Gelidium abbottiorum	NAT3768-3770	Norris	NU
KZN	Umkomas/Widenham	Gelidium abbottiorum	NAT3768	Norris	NU
KZN	Umkomas/Widenham	Gelidium abbottiorum	NAT3768	Norris	NU
KZN	Umdoni Park	Gelidium amansii	8514	Jarman & Guy	NU
KZN	Rocky Bay	Gelidium abbottiorum	002701 / NAT470	Norris	NU

KZN	Rocky Bay, Park Rynie	Gelidium amansii	NAT1129	Norris	WITS
KZN	Pennington	Gelidium amansii	NAT5507	Wadge	WITS
KZN	Ifafa Beach	Gelidium abbottiorum	3698	Norris	NU
KZN	Umtentweni	Gelidium abbottiorum	010559 / NAT2334	Norris	NU
KZN	Umtentweni	Gelidium abbottiorum	SAM100332/ NAT2334	Norris	NU
KZN	Umtentweni	Gelidium abbottiorum	I34374	Isaac	BOL
KZN	St. Michaels-on-Sea	Gelidium abbottiorum	I34378	Isaac	BOL
KZN	Southbroom	Gelidium abbottiorum	SAM100337/ NAT5657	Norris	NU
KZN	Palm Beach	Gelidium abbottiorum	KZN811	Bolton et al.	BOL/GENT
KZN	Palm Beach	Gelidium abbottiorum	KZN832B	Bolton	BOL/GENT
KZN	Palm Beach	Gelidium abbottiorum	678	Norris	NU
KZN	Glenmore Beach	Gelidium amansii	6664	van der Schijff	NU
EC	Mzamba Beach	Gelidium abbottiorum	901	R. H. Simons	BOL
EC	Mbotyi	Gelidium abbottiorum		McKay 2000: pers. comm	
EC	Manteku	Gelidium abbottiorum		McKay 2000: pers. comm	
EC	Ntafufu	Gelidium abbottiorum	TRN1	Tronchin	BOL
EC	Ntafufu	Gelidium abbottiorum	TRN2	Tronchin	BOL
EC	Port St. Johns	Gelidium amansii	989	R. H. Simons	
EC	Hluleka	Gelidium arenarium		Bolton and Stegenga (1987)	
EC	Breezy Pt. (The Haven)	Gelidium abbottiorum	Q2/3/4/5/6/7	Tronchin	BOL
EC	Bashee River mouth	Gelidium rigidum	1663	M. A. Pocock	GRA
EC	Bashee River mouth	Gelidium abbottiorum	1648	Norris	NU
EC	Dwessa	Gelidium abbottiorum	sn5214	Simons	BOL
EC	Dwessa	Gelidium capense	sn5215	Simons	BOL
EC	Qolora	Gelidium abbottiorum	I34381	Isaac	BOL
EC	Kei Mouth (Wacky Pt.)	Gelidium abbottiorum	1a	Tronchin	BOL
EC	Kei Mouth (Wacky Pt.)	Gelidium abbottiorum	GSA5	Tronchin	BOL
EC	Cape Morgan	Gelidium abbottiorum	25624	Norris	NU
EC	Cape Morgan	Gelidium abbottiorum	25623	Norris	NU
EC	Cape Morgan	Gelidium abbottiorum	2a	Tronchin	BOL
EC	Cape Morgan	Gelidium abbottiorum	GSA6	Tronchin	BOL
EC	Double Mouth	Gelidium abbottiorum	3a	Tronchin	BOL
EC	Double Mouth	Gelidium abbottiorum	3a	Tronchin	BOL
EC	Double Mouth	Gelidium abbottiorum	GSA7	Tronchin	BOL
EC	Haga Haga	Gelidium abbottiorum	4a	Tronchin	BOL
EC	Haga Haga	Gelidium abbottiorum	GSA8	Tronchin	BOL
EC	Glengariff	Gelidium abbottiorum	sn5294	Simons	BOL
EC	Gonubie Point	Gelidium rigidum	1891	M. A. Pocock	GRA
EC	Gonubie Point	Gelidium abbottiorum	SAM103127/ NAT6536	P. van Zyl	NU
EC	East London - Bats Cave	Gelidium amansii	SAM 100419		NU
EC	East London - Bats Cave	Gelidium amansii	8532	M. A. Pocock	GRA
EC	East London - Bats Cave	Gelidium abbottiorum	SAM105565	Norris	NU
EC	Nahoon Reef	Gelidium abbottiorum	P38	Tronchin	BOL
EC	East London - Fuller's Beach	Gelidium amansii	7258	M. A. Pocock	GRA
EC	Kidds Beach	Gelidium abbottiorum	675	Norris	NU
EC	Fish River Mouth	Gelidium amansii	31833	B. Sonnenberg	GRA
EC	Riet Rivier - Black Rock	Gelidium amansii	29334	E. Olivieri	GRA
EC	Riet Rivier - Black Rock	Gelidium versicolor	10	T. Everett	GRA
EC	Riet Rivier - Black Rock	Gelidium abbottiorum	5a	Tronchin	BOL
EC	Kowie	Gelidium abbottiorum	333	Tyson	NU
EC	Port Alfred / The Kowie	Gelidium rigidum	2385a	M. A. Pocock	GRA
EC	Port Alfred - Sharks Bay	Gelidium amansii	13619	M. A. Pocock	GRA
EC	Port Alfred - Sharks Bay	Gelidium abbottiorum	26033	Norris	NU
EC	Port Alfred - Soutvleibaai	Gelidium abbottiorum	sn5069	Simons	BOL
EC	Port Alfred - Soutvleibaai	Gelidium abbottiorum	6a	Tronchin	BOL
EC	Port Alfred - Piano Rocks	Gelidium abbottiorum	TRN7	Tronchin	BOL
EC	Port Alfred - Piano Rocks	Gelidium abbottiorum	TRN8	Tronchin	BOL

EC	Port Alfred - Piano Rocks	Gelidium abbottiorum	6b	Tronchin	BOL
EC	Kenton on Sea	Gelidium amansii	31811	M. D. Stobart	GRA
EC	Kenton on Sea	Gelidium abbottiorum	sn5107	Hewitt	BOL
EC	Bushman's River Mouth	Gelidium amansii	36730	G. B. Hellstrom	GRA
EC	Bushman's River Mouth	Gelidium amansii	31739	G. van Heerden	GRA
EC	Bushman's River Mouth	Gelidium amansii	31382	M. Lipskey	GRA
EC	Bushman's River Mouth	Gelidium rigidum	6009	M. A. Pocock	GRA
EC	Cannon Rock	Gelidium abbottiorum		McKay 2000: pers. comm	
EC	Bird Island	Gelidium amansii		Anderson and Stegenga (1989)	
EC	Seaview, Port Elizabeth	Gelidium abbottiorum		McKay 2000: pers. comm	
EC	Cape St Francis	Gelidium abbottiorum		McKay 2000: pers. comm	
WC	Robberg (Plettenburg Bay)	Gelidium abbottiorum	11416	Isaac	BOL
WC	Blauwklip (Knysna)	Gelidium asperum	A.V.D.8010		NU
WC	Buffalo Bay	Gelidium asperum	18	A. Duthie	BOL
WC	Tergniet	Gelidium abbottiorum	5059	Isaac	BOL
WC	Preekstoel (Stilbaai)	Gelidium abbottiorum	sn1583	Simons	BOL
WC	De Hoop Nature Reserve	Gelidium abbottiorum		Bolton and Stegenga (1990)	
WC	Arniston	Gelidium rigidum	6609a&e	M. A. Pocock	GRA
WC	Arniston	Gelidium rigidum	3082b	M. A. Pocock	GRA
WC	Arniston	Gelidium abbottiorum	2877	Norris	NU
WC	L'Agulhas	Gelidium rigidum	1358	M. A. Pocock	GRA
WC	Brandfontein	Gelidium abbottiorum	sa2773	Anderson et al.	BOL
WC	Brandfontein	Gelidium abbottiorum	WC1	Tronchin	BOL
WC	Hangklip	Gelidium abbottiorum		Jackelman et al. (1991)	
WC	McDougall's Bay	Gelidium abbottiorum	sn5188	F.E. Hewitt	BOL

Gelidium applanatum

WC	Muizenberg (drift, on Ecklonia)	Gelidium applanatum		Stegenga et al. (1997)	
WC	Vulcan Rock	Gelidium applanatum	sa2968	Anderson	BOL

Gelidium arenarium

KZN	Jesser Point, Sodwana	Pterocladiella caespitosa	P18	Tronchin	BOL
KZN	Mvoti	Gelidium arenarium	100420 / NAT2449	Norris	NU
KZN	Isipingo	Gelidium arenarium	02/0170441	T.A.Stephenson	LD
KZN	Isipingo	Gelidium arenarium	SAM 100422	T.A.Stephenson	NU
EC	Hluleka	Gelidium arenarium		Bolton and Stegenga (1987)	
EC	Cape Morgan	Gelidium sp.	7078	Pocock	GRA
EC	Waterloo Bay	Gelidium sp.	8099	Pocock	GRA
EC	Waterloo Bay	Gelidium sp.	8164	Pocock	GRA
EC	Fish Point	Gelidium sp.	8031b	Pocock	GRA
EC	Kowie	Gelidium sp.	1376b	Pocock	GRA
EC	Bushmans River	Gelidium sp.	6017	Pocock	GRA
EC	Cannon Rocks	Gelidium sp.	8224	Pocock	GRA
EC	Jeffreys Bay	Gelidium sp.	8310	Pocock	GRA
EC	Groot Rivier	Gelidium?	6443	Pocock	GRA
EC	Groot Rivier	Gelidium sp.	6443/6445	Pocock	GRA
WC	Plettenberg Bay	Gelidium sp.	645b	Pocock	GRA
WC	Buffalo bay	Gelidium sp.	7884	Pocock	GRA
WC	Great Brak River	Gelidium sp.	392	Pocock	GRA
WC	Great Brak River	Gelidium	392	Pocock	GRA
WC	Tergniet	Gelidium arenarium	1328	Isaac	BOL
WC	Arniston	Gelidium	603	Pocock	GRA
WC	Arniston	Gelidium	606	Pocock	GRA
WC	Arniston	unnamed	292	Pocock	GRA
WC	Arniston	Gelidium sp.	603	Pocock	GRA
WC	Arniston	Gelidium sp.	2897a	Pocock	GRA

WC	Arniston	Gelidium sp.	606	Pocock	GRA
WC	Arniston	Gelidium sp.	1242b	Pocock	GRA
WC	Arniston	Gelidium sp.	2891a	Pocock	GRA
WC	Arniston	Gelidium sp.	2893a,-94a,-95a,-96a	Pocock	GRA
WC	Swartklip	Gelidium sp.	3492	Pocock	GRA
WC	Muizenberg	Gelidium arenarium	100423	Papenfuss	NU

Gelidium capense

KZN	Palm Beach	Gelidium capense	8502	Norris	NU
EC	Cape Morgan	Gelidium	7040	Pocock	GRA
EC	Cape Morgan	Gelidium cartilagineum	283/831	H. G. Flanagan	NU
EC	Gonubie Point	Gelidium capense	SAM103129	P. van Zyl	NU
EC	East London	Gelidium capense	8504	Norris	NU
EC	East London	Gelidium cartilagineum	9074	Pocock	GRA
EC	Riet Rivier - Black Rock	Gelidium cartilagineum	7492	Pocock	GRA
EC	Port Alfred/ The Kowie	Gelidium capense	37968	Becker	BOL
EC	Port Alfred/ The Kowie	Gelidium capense	9606	Norris	NU
EC	Groot Rivier	Gelidium cartilagineum	6426	Pocock	GRA
EC	Storms River Mouth	Gelidium cartilagineum	6595	Pocock	GRA
WC	Heralds Bay	Gelidium sp.	8285		NU
WC	De Hoop Nature Reserve	Gelidium capense		Bolton and Stegenga (1990)	
WC	Kaap Infante	Gelidium capense	sa615	Stegenga	NU
WC	Arniston	Gelidium capense	3772	Norris	NU
WC	Cape Agulhas	Gelidium capense	sa2623	Stegenga	BOL
WC	Brandfontein	Gelidium capense	sc2774	Stegenga	BOL
WC	West Cliffs (Hermanus)	Gelidium capense	62	M. R. Michell	BOL
WC	Betty's Bay	Gelidium capense	3629	Norris	NU
WC	Hangklip	Gelidium capense		Jackelman et al. (1991)	
WC	Muizenberg	Gelidium capense	sa217	Stegenga	BOL
WC	St. James	Gelidium capense	sa79	Stegenga	BOL
WC	Dalebrook	Gelidium capense	8502	Norris	NU
WC	Clovelly	Gelidium capense	WC4	Tronchin	BOL
WC	Glencairn	Gelidium capense	GSA10	Tronchin	BOL
WC	Glencairn	Gelidium capense	GSA11	Tronchin	BOL
WC	Glencairn	Gelidium capense	GSA12	Tronchin	BOL
WC	Glencairn	Gelidium capense	GSA13	Tronchin	BOL
WC	Glencairn	Gelidium capense	GSA14	Tronchin	BOL
WC	Glencairn	Gelidium pteridifolium	sa2833	Stegenga	BOL
WC	Glencairn	Gelidium capense	WC2	Tronchin	BOL
WC	Glencairn	Gelidium capense	WC3	Tronchin	BOL
WC	Oatlands Point	Gelidium capense	SAM100199	J. Scott	NU
WC	Miller's Point	Gelidium pteridifolium	2490	Simons	BOL
WC	Buffelsbaai	Gelidium capense	WC5	Tronchin	BOL
WC	Platboombaai	Gelidium capense	sa477	Stegenga	BOL
WC	Kommetjie	Gelidium capense	WC6	Tronchin	BOL
WC	Oude Schip	Gelidium capense	GSA1	Tronchin	BOL
WC	Oudekraal	Gelidium capense	WC7	Tronchin	BOL
WC	Bakoven	Gelidium capense	2528	Simons	BOL
WC	Camps Bay	Gelidium capense	GSA2a	Tronchin	BOL
WC	Camps Bay	Gelidium capense	GSA2b	Tronchin	BOL
WC	Camps Bay	Gelidium capense	GSA3	Tronchin	BOL
WC	Camps Bay	Gelidium capense	GSA4	Tronchin	BOL
WC	Table bay	Gelidium capense	37974 / 37978	Tyson	BOL
WC	Table bay	Gelidium cartilagineum	25618	Tyson	NU
WC	Paternoster	Gelidium capense	sn470	Simons	BOL
WC	Saldanha Bay	Gelidium pteridifolium	422 / 424	Schils (1998)	GENT

Gelidium crinale

KZN	St Lucia Rocks	Pterocladia heteroplatos	2114a	Norris	GRA
EC	Cape Morgan	Gelidium arenarium	36576	Flanagan	BOL
EC	Cape Morgan	Gelidium elminense	36575	Norris	BOL
EC	Cape Morgan	Gelidium sp.	1819	Pocock	GRA
EC	Waterloo Bay	Gelidium sp.	8067	Pocock	GRA
EC	Kowie	Gelidium sp.	2040	Pocock	GRA
EC	Cannon Rocks	Gelidium sp.	7988	Pocock	GRA
WC	Kalk Bay	G. crinale	SAM105059		NU

Gelidium declerckianum

EC	Nahoon Reef (East London)	Onikusa foliacea	99067	Tronchin	BOL
EC	Kidds Beach	Gelidium arenarium	110878	Tronchin	BOL

Gelidium foliaceum

KZN	Sodwana Bay	Onikusa foliacea	SAM102180 / NAT6183	Norris	NU
KZN	Mvoti	Onikusa foliacea	SAM100418 / NAT1626	Norris	NU
KZN	Umdhloti	Onikusa foliacea	3123	Norris	NU
KZN	Trafalgar	Onikusa foliacea	SAM103232 / NAT5713	Norris	NU
KZN	Palm Beach	Onikusa foliacea	SAM100415 / NAT4750	Norris	NU
KZN	Palm Beach	Onikusa foliacea	SAM103104 / NAT6487	Norris	NU
KZN	Port Edward	Onikusa foliacea	P14	Tronchin	BOL
EC	Manteku	Onikusa foliacea	TRNB	Tronchin	BOL
EC	Breezy Point	Onikusa foliacea	S19	Tronchin	BOL
EC	Kei Mouth, Wacky Pt.	Onikusa foliacea	1d	Tronchin	BOL

Gelidium isabelae

EC	Skoenmakerskop, PE	Gelidium isabelae	5221	Simons	BOL
WC	Swartklip	Gelidium isabelae		Freshwater and Millar (2004)	

Gelidium micropterum

KZN	Isipingo	Gelidium micropterum	9927	Pocock	GRA
KZN	Southbroom	Gelidium sp. nov.	NAT1875	Vincent	WITS
KZN	Palm Beach	Gelidium micropterum	NAT4007	Norris	WITS
EC	Double Mouth	Gelidium micropterum	3h	Tronchin	BOL
WC	Hangklip	Gelidium micropterum	Sa2214	Bolton	BOL
WC	St. James	Gelidium micropterum	SAM103240	Norris	NU
WC	Clovelly	Gelidium micropterum	Sa2251	Stegenga	BOL
WC	Glencairn	Gelidium micropterum	Sa2031	Stegenga	BOL
WC	Platboom	Gelidium micropterum	11958	Pocock	GRA
WC	Kommetjie	Gelidium micropterum	2345	Simons	BOL
WC	Oude Schip	Gelidium micropterum	4598	Pocock	GRA
WC	Sea Point	Gelidium micropterum	9605	Norris	NU
WC	3 Anchor Bay	Gelidium micropterum	4797	Pocock	GRA
WC	Paternoster	Gelidium micropterum	462	Simons	BOL
NC	Buffels River	Gelidium micropterum	1952	Papenfuss	BOL

Gelidium minusculum

KZN	Rocky Bay	Gelidium minusculum	SAM100434/ NAT2440	Pienaar	NU
KZN	Rocky Bay	Gelidium minusculum	SAM100435/ NAT2440	Pienaar	NU

Gelidium pristoides

KZN	Ouma's Pool, Port Edward	Gelidium pristoides	NAT7165		NU
EC	Manteku	Gelidium pristoides		Mckay 2000: pers. comm.	

EC	Mntafufu	Gelidium pristoides	TRN4	Tronchin	
EC	Dome Bluff	Gelidium pristoides	TRN5	Tronchin	BOL
EC	Port St. Johns	Suhria pristoides	sn975	Simons	BOL
EC	Hluleka north	Gelidium pristoides	T117	Stegenga	BOL
EC	Presley's Bay	Suhria pristoides	T199	Stegenga	BOL
EC	Breezy Point	Gelidium pristoides	T17/S17	Tronchin	BOL
EC	Bashee River mouth	Gelidium pristoides	1667	Pocock	GRA
EC	Dwessa	Suhria pristoides	sn5198	Simons	BOL
EC	Centani coast	Suhria pristoides	34417	Isaac	BOL
EC	Kei River mouth	Gelidium pristoides	9826		NU
EC	Kei Mouth (Wacky Pt.)	Gelidium pristoides	GSA5	Tronchin	BOL
EC	Kei Mouth (Wacky Pt.)	Onikusa pristoides	1c	Tronchin	BOL
EC	Cape Morgan	Gelidium pristoides	25613	Tyson	BOL
EC	Cape Morgan	Gelidium pristoides	GSA6	Tronchin	BOL
EC	Cape Morgan	Gelidium pristoides	3788	Pocock	GRA
EC	Double Mouth	Gelidium pristoides	GSA7	Tronchin	BOL
EC	Double Mouth	Onikusa pristoides	3c	Tronchin	BOL
EC	Haga Haga	Gelidium pristoides	GSA8	Tronchin	BOL
EC	Haga Haga	Onikusa pristoides	4c	Tronchin	BOL
EC	Haga Haga	Gelidium pristoides	3628	Pocock	GRA
EC	Gonubie Point	Gelidium pristoides	1894a	Pocock	GRA
EC	Gonubie	Gelidium pristoides	REN8155		NU
EC	Nahoon Reef	Gelidium micropterum	P35/S15	Tronchin	BOL
EC	Nahoon Reef	Gelidium pristoides turf	P35/S15	Tronchin	BOL
EC	East London	Suhria pristoides	1813		BOL
EC	East London	Gelidium pristoides	8505		NU
EC	Kidds Beach	Gelidium pristoides turf	P39/S16	Tronchin	BOL
EC	Hamburg (Keiskammapunt)	Gelidium pristoides	13933	Pocock	GRA
EC	Hamburg (Keiskammapunt)	Suhria pristoides	34420	Isaac	BOL
EC	Chalumna	Gelidium pristoides	REN7740	Pienaar	NU
EC	Kleinemonde	Gelidium pristoides	7424	Pocock	GRA
EC	Three Sisters	Onikusa pristoides	5c	Tronchin	BOL
EC	Port Alfred/Kowie	Gelidium pristoides	632	Pocock	GRA
EC	Port Alfred/Kowie	Suhria pristoides	34412	Becker	BOL
EC	Kowie	Gelidium pristoides	8977		NU
EC	Port Alfred	Gelidium pristoides	SAM98454	Tyson	NU
EC	Bushman's River Mouth	Gelidium pristoides	1634	J. L. Gordon-Gray	NU
EC	Cannon Rocks	Gelidium pristoides	NU/290/95	H. Stadion	NU
EC	Bird Island	Gelidium pristoides		Anderson and Stegenga (1989)	
EC	Beacon Pt. (Port Elizabeth)	Suhria pristoides	sn5071	Simons	BOL
EC	Jeffreys Bay	Gelidium pristoides	NU/261/95		NU
EC	Groot Rivier	Gelidium pristoides	6425	Pocock	GRA
EC	Storms River Mouth	Gelidium pristoides	6588	Pocock	GRA
WC	Nature's Valley	Gelidium pristoides	694		NU
WC	Nature's Valley	Gelidium pristoides	694		NU
WC	Blauwklip (Knysna)	Gelidium pristoides	NU/302/95	H. C. Taylor	NU
WC	Buffelsbaai	Gelidium pristoides	4699	Pocock	GRA
WC	Walker Bay	Suhria pristoides	sn4322	Simons	BOL
WC	Kaap Infante - St Sebastian Pt	Suhria pristoides	Sa617	Stegenga	BOL
WC	Waenhuiskrans - nr. Arniston	Gelidium pristoides	NU/305/95	H. Stadion	NU
WC	Arniston	Gelidium pristoides	544	Pocock	GRA
WC	Arniston	Gelidium pristoides	SAM103212		NU
WC	Arniston	Gelidium sp.	1235	Pocock	GRA
WC	Agulhas	Gelidium pristoides	SAM103213		NU
WC	Brandfontein	Suhria pristoides	Sa2703	Stegenga	BOL
WC	Stanford Beach (Gansbaai)	Gelidium pristoides	3851		NU
WC	Voelklip (Hermanus)	Gelidium pristoides	2385	B. A. Louwrens	NU

WC	Sandbaai	Gelidium pristoides	2550	B. A. Louwrens	NU
WC	Betty's Bay	Gelidium pristoides	691		NU
WC	Pringle Bay	Gelidium pristoides	454	Pocock	GRA
WC	Gordons Bay	Gelidium pristoides	NU/282/95		NU
WC	Somerset Strand	Gelidium pristoides	2975	Pocock	GRA
WC	Somerset Strand	Gelidium pristoides	2975	Pocock	GRA
WC	Eersterivier	Suhria pristoides	34417	Isaac	BOL
WC	Swartklip	Gelidium pristoides	3526	Pocock	GRA
WC	Strandfontein	Gelidium pristoides	1408	Pocock	GRA
WC	Strandfontein	Gelidium pristoides	7886		NU
WC	False Bay	Gelidium pristoides	25615	Tyson	BOL
WC	False Bay to Kei River	Gelidium pristoides	90	Tyson	NU
WC	False Bay	Gelidium pristoides	1024	Tyson	NU
WC	St. James	Gelidium pristoides	693		NU
WC	St. James	Gelidium pristoides	798	Pocock	GRA
WC	St. James	Suhria pristoides	Sa41	Stegenga	BOL
WC	Dalebrook	Gelidium pristoides	735	Pocock	GRA
WC	Dalebrook, False Bay, Cape	Gelidium pristoides	8460		NU
WC	Kalk Bay	Gelidium pristoides	4765	Pocock	GRA
WC	Kommetjie	Suhria pristoides	sn65	Simons	BOL
WC	Melkbosstrand	Gelidium pristoides	343	Pocock	GRA

Gelidium profundum

KZN	Protea Banks (Shelly Beach)	Gelidium profundum	Sp31	Tronchin	BOL
KZN	Protea Banks, N. Edge	Gelidium profundum	P8	Tronchin	BOL
KZN	Protea Banks, N. Edge	Gelidium profundum	P11	Tronchin	BOL
KZN	Protea Banks, N. Edge	Gelidium profundum	ET19	Tronchin	BOL
KZN	Protea Banks, N. Edge	Gelidium profundum	ET20	Tronchin	BOL

Gelidium pteridifolium

KZN	Tinley Manor	Gelidium pteridifolium	NAT6094	Clapham	WITS
KZN	Umdloti - Sentinel Rocks	Gelidium pteridifolium	sn705	Simons	BOL
KZN	Bluff	Gelidium pteridifolium	2333	Pocock	GRA
KZN	Reunion Rocks	Gelidium pteridifolium	9726 / NAT1831	Norris	NU
KZN	Isipingo	Gelidium pteridifolium	196		NU
KZN	Isipingo	Gelidium pteridifolium	15A / 203		NU
KZN	Isipingo	Gelidium pteridifolium	8513	S. Seagrief	NU
KZN	Tiger Rocks	Gelidium pteridifolium	SAM100250/ NAT6144	F. Junor	NU
KZN	Umkomas - Widenham	Gelidium pteridifolium	10560	Norris	NU
KZN	Rocky Bay, Park Rynie	Gelidium pteridifolium	NAT1143	Norris	WITS
KZN	Ifafa Beach	Gelidium pteridifolium	3710	L. Rushworth	NU
KZN	Uvongo	Gelidium cartilagineum	10063	Pocock	GRA
KZN	Uvongo	Gelidium cartilagineum	2134	A.O.D. Mogg	NU
KZN	Southbroom	Gelidium pteridifolium	SAM104118/ NAT5682	L. Rushworth	NU
KZN	Marina Beach	Gelidium pteridifolium	007333 / NAT1375	Norris	NU
KZN	Trafalgar	Gelidium pteridifolium	SAM102301/ NAT6513	Norris	NU
KZN	Palm Beach	Gelidium pteridifolium	007113 / NAT1143	M.E. Aken	NU
KZN	Palm Beach	Gelidium pteridifolium	ET10	Tronchin	BOL
KZN	Palm Beach	Gelidium pteridifolium	Sp21	Tronchin	BOL
KZN	Port Edward	Gelidium pteridifolium	Sp21	Tronchin	BOL
EC	Mzamba Beach	Gelidium pteridifolium	sn900	Simons	BOL
EC	Ntafufu	Gelidium pteridifolium	TRN2	Tronchin	BOL
EC	Ntafufu	Gelidium pteridifolium	TRN3	Tronchin	BOL
EC	Port St. Johns	Gelidium pteridifolium	sn964	Simons	BOL
EC	Hluleka	Gelidium pteridifolium	T51	Stegenga	BOL
EC	Presley's Bay	Gelidium pteridifolium	T184	Stegenga	BOL
EC	Breezy Pt. (The Haven)	Gelidium pteridifolium	Q1/4/8/9	Tronchin	BOL

EC	Bashee River mouth	Gelidium cartilagineum	1662	Pocock	GRA
EC	Kei Mouth (Wacky pt.)	Gelidium pteridifolium	1b	Tronchin	BOL
EC	Kei Mouth (Wacky pt.)	Gelidium pteridifolium	GSA5	Tronchin	BOL
EC	Cape Morgan	Gelidium pteridifolium	034384/5	Tyson	BOL
EC	Cape Morgan	Gelidium pteridifolium	2b	Tronchin	BOL
EC	Cape Morgan	Gelidium pteridifolium	GSA6	Tronchin	BOL
EC	Double Mouth	Gelidium pteridifolium	3b	Tronchin	BOL
EC	Double Mouth	Gelidium pteridifolium	GSA7	Tronchin	BOL
EC	Haga Haga	Gelidium pteridifolium	4b	Tronchin	BOL
EC	Haga Haga	Gelidium pteridifolium	GSA8	Tronchin	BOL
EC	Gonubie	Gelidium pteridifolium	NAT6538	van Zyl	NU
EC	East London	Gelidium cartilagineum	6505	Pocock	GRA
EC	East London - Cove Rock	Gelidium pteridifolium	sn1182	Simons	BOL
EC	Kidds Beach	Gelidium pteridifolium	134367	Isaac	BOL
EC	Riet Rivier - Black Rock	Gelidium rigidum	6939	M. A. Pocock	GRA
EC	Riet Rivier - Black Rock	Gelidium pteridifolium	5b	Tronchin	BOL
EC	Port Alfred/ The Kowie	Gelidium cartilagineum	2386	Pocock	GRA
EC	Port Alfred/ The Kowie	Gelidium pteridifolium	9737	Becker	NU
EC	Kasouga	Gelidium pteridifolium	10421	B.J. White	NU
EC	Kenton-on-Sea	Gelidium pteridifolium	sn5093	Simons	BOL
EC	Bushman's River Mouth	Gelidium cartilagineum	2006C	E. L. Stephens	NU
EC	Bird Island	Gelidium pteridifolium		Anderson and Stegenga (1989)	
EC	Storms River Mouth	Gelidium cartilagineum	33294	E. M. Donald	GRA
EC	Storms River Mouth	Gelidium cartilagineum	6595	Pocock	GRA
WC	DeHoop Nature Reserve	Gelidium capense	sa587	Stegenga	BOL
WC	Agulhas Beach	Gelidium cartilagineum	8175		NU
WC	Muizenberg	Gelidium pteridifolium	25965	E. K. Fredgold	NU
WC	Table bay	Gelidium capense	37971	Tyson	BOL
Gelidium reptans					
KZN	Bhanga Neck, Boteler Point	Gelidium reptans	KZN121	Tronchin	BOL/GENT
KZN	Sodwana Point	Gelidium reptans	P20	Tronchin	BOL
KZN	St. Lucia - Cape Vidal	Gelidium reptans	100430 / NAT5241	F. Molloy	NU
KZN	Umvoti Mouth	Gelidium reptans	008330 / NAT1626	Norris	NU
KZN	Umvoti intertidal	Gelidium reptans	SAM100424/NAT1626a	Norris	NU
KZN	Umvoti intertidal	Gelidium reptans	NU008638 / NAT1626	Norris	NU
KZN	Sheffield Beach	Gelidium reptans	2602	Simons	BOL
KZN	Shaka's Rock	Gelidium reptans	KZN188	Tronchin	BOL/GENT
KZN	Umdloti - Sentinel Rocks	Gelidium reptans	SAM100417 / NAT5695	Norris	NU
KZN	Umdloti - Sentinel Rocks	Gelidium reptans	SAM102060 / NAT6185	Norris	NU
KZN	Umhlanga Rocks	Gelidium reptans	2336	Pocock	GRA
KZN	Treasure Beach	Gelidium reptans	KZN119	Tronchin	BOL/GENT
KZN	Reunion Rocks	Gelidium reptans	009218 / NAT1795	Norris	NU
KZN	Reunion Rocks	Gelidium reptans	SAM100429 / NAT1795	Aken	NU
KZN	Isipingo	Gelidium reptans	SAM100428	T. A. Stephenson	NU
KZN	Isipingo	Gelidium reptans	59	Forbes	NU
KZN	Rocky Bay, Park Rynie	Pterocladia caespitosa	10590	Pienaar	NU
KZN	Rocky Bay	Gelidium reptans	SAM100427/ NAT1423	Norris	NU
KZN	Rocky Bay	Gelidium reptans	007357 / NAT1423	Norris	NU
KZN	Hibberdene	Gelidium reptans	10024	Pocock	GRA
KZN	Shelly Beach (Protea Banks)	Gelidium reptans	7893		NU
EC	Hluleka	Gelidium reptans		Bolton and Stegenga (1987)	
EC	Qora	Gelidium sp.	2071	Pocock	GRA
EC	Cape Morgan	Gelidium reptans	1866	Pocock	GRA
EC	Double Mouth	Gelidium reptans	3f	Tronchin	BOL

EC	Double Mouth	Gelidium reptans	3d	Tronchin	BOL
EC	Double Mouth	Gelidium reptans	3e	Tronchin	BOL
EC	Haga Haga	Gelidium sp.	3634	Pocock	GRA
EC	Three Sisters	Gelidium reptans	5d	Tronchin	BOL
EC	Riet Rivier - Black Rock	Gelidium reptans	7479	Pocock	GRA
WC	De Hoop Nature Reserve	Gelidium reptans		Bolton and Stegenga (1990)	
WC	Arniston	Gelidium sp.	3089a	Pocock	GRA
WC	Hangklip to Agulhas	Gelidium reptans		Engledow (1998)	
WC	False Bay	Gelidium reptans		Engledow (1998)	
WC	Cape of Good Hope	Phyllophora reptans	33266	Suhr	LD

Gelidium sp.

KZN	Sodwana (9-Mile reef)	Gelidium?	P25	Tronchin	BOL
KZN	Sodwana (2-Mile reef)	Pterocladiella caerulescens	KZN265B	Bolton et al.	BOL

Gelidium vittatum

EC	Seaview (Port Elizabeth)	Suhria vittata	8419	Pocock	GRA
WC	Natures Valley	Suhria vittata	1126	E.G. Akhurst	NU
WC	Whitesands (Brede River)	Suhria vittata	none		NU
WC	Cape Agulhas	Suhria vittata	none		NU
WC	Pearly Beach	Suhria vittata	none		BOL
WC	Franskraal	Suhria vittata	2608	Simons	NU
WC	Voelklip, Hermanus	Suhria vittata	2403	B. Louwrens	NU
WC	Betty's Bay	Suhria vittata	1125		NU
WC	Hangklip, Skuifbaai	Suhria vittata	Sa2181	Stegenga	BOL
WC	Somerset Strand	Suhria vittata	873	Mej. H.J. Smit	NU
WC	Swartklip, East	Suhria vittata	4193a	Pocock	GRA
WC	Muizenberg Beach	Suhria vittata	SAM 98391	A.G. Mcloughlin	NU
WC	Muizenberg Beach	Suhria vittata	956	Tyson	NU
WC	Platboombaai, Cape Pt.	Suhria vittata	Sa493	Stegenga	BOL
WC	Olifantsbosch, Cape Pt.	Suhria vittata	Sa165	Stegenga	BOL
WC	Slangkop Pt. C.P	Suhria vittata	SAM 98487		NU
WC	Kommetjie	Suhria vittata	GSA9	Tronchin	BOL
WC	Kommetjie	Suhria vittata	8553	M.E. Aken	NU
WC	Kommetjie	Suhria vittata	Sa233	Stegenga	BOL
WC	Oudekraal	Suhria vittata	GSA15	Tronchin	BOL
WC	Bakoven	Suhria vittata	2527	Simons	BOL
WC	Camps Bay	Suhria vittata	957	Tyson	NU
WC	Sea Point	Suhria vittata	954	Flanagan	NU
WC	3-Anchor Bay	Suhria vittata	none	M.H. Louw	NU
WC	Mouille Point	Suhria vittata	Sa2918	Stegenga	BOL
WC	Table Bay	Suhria vittata	955	Tyson	NU
WC	False Bay	Suhria vittata	44	Tyson	NU
WC	Yzerfontein	Suhria vittata	Sa2131	Stegenga	BOL
WC	Cape Hondeklipbaai	Suhria vittata	Sa2491	Stegenga	BOL
WC	Lambertsbaai	Suhria vittata	Sa800	Stegenga	BOL
WC	Strandfontein - Olifants River	Suhria vittata	2476	Pocock	BOL
WC	Peacock's Bay	Suhria vittata	269	Simons	BOL

Pterocladiella bartlettii

KZN	Jesser Pt., Sodwana	Pterocladiella bartlettii	P22	Tronchin	BOL

Pterocladiella 'caerulescens'

KZN	Mabibi	Pterocladiella caerulescens	P30	Tronchin	BOL
KZN	Mabibi	Pterocladiella caerulescens	P31	Tronchin	BOL
KZN	Sodwana (9-Mile reef)	Pterocladiella caerulescens	P24	Tronchin	BOL

Pterocladiella caespitosa

KZN	Mabibi, The Ledges	Pterocladiella caespitosa	KZN484B	Bolton et. al.	BOL/GENT
KZN	Sodwana	Pterocladia caespitosa	NAT 6317		NU
KZN	St. Lucia Rocks	Pterocladia caespitosa	SAM100451		NU
KZN	Mvoti	Pterocladia caespitosa	NAT1664	Norris & Aken	WITS
KZN	Umdloti	Pterocladia caespitosa	SAM100444		NU
KZN	Umdloti beach	Pterocladia caespitosa	NAT1424 / 006286		NU
KZN	Durban	Gelidium caespitosum	02/0170440	T.A. Stephenson	LD
KZN	Isipingo	Pterocladia caespitosa	2343	Norris	GRA
KZN	Isipingo	Gelidium caespitosum	9932	Pocock	GRA
KZN	Reunion Rocks	Gelidium caespitosum	9965	Pocock	GRA
KZN	Reunion Rocks	Pterocladia caespitosa	NAT1882		NU
KZN	Treasure Beach	Pterocladia caespitosa	HEC10989	Coppejans	GENT
KZN	Rocky Bay, Park Rynie	Pterocladia caespitosa	NAT1425		NU
KZN	Rocky Bay, Park Rynie	Pterocladia caespitosa	10590	Norris	NU
EC	Hluleka	Pterocladia caespitosa		Bolton and Stegenga (1987)	

Pterocladiella caloglossoides

KZN	Isipingo	Pterocladia caloglossoides		Farrel *et. al.* (1993)	
KZN	Reunion Rocks	Pterocladia caloglossoides	NAT2047		NU
KZN	Rocky Bay, Park Rynie	Pterocladia caloglossoides	NAT2426		NU
WC	Strandfontein	Pterocladiella caloglossoides	1052	Tronchin	GRA

Pterocladiella sp.

KZN	Sodwana (2 mile reef)	Pterocladiella caerulescens	P17	Tronchin	BOL
KZN	Sodwana (2 mile reef)	Pterocladiella caerulescens	P21	Tronchin	BOL

Ptilophora coppejansii

KZN	Protea Banks	Ptilophora coppejansii	99070	Tronchin	BOL
KZN	Protea Banks	Ptilophora coppejansii	511	Tronchin	BOL
KZN	Protea Banks, Salmon Bank	Ptilophora coppejansii	KZN1992	Tronchin	GENT

Ptilophora diversifolia

KZN	Widenham	Ptilophora diversifolia	1525	Norris	NU
KZN	Park Rynie: Lander's Green	Ptilophora diversifolia	NAT 2711	Norris	NU
KZN	Protea Banks	Ptilophora diversifolia	Sp 30	Tronchin	BOL
KZN	Protea Banks, N. Edge	Ptilophora diversifolia	ET17	Tronchin	BOL
KZN	Protea Banks, N. Edge	Ptilophora diversifolia	ET18	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora diversifolia	P3	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora diversifolia	ET14	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora diversifolia	P5	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora diversifolia	ET15	Tronchin	BOL
KZN	Marina Beach	Ptilophora diversifolia	7316	Norris	NU
KZN	Palm Beach	Ptilophora diversifolia	NAT 1278	Norris	NU
KZN	Palm Beach	Ptilophora diversifolia	9650	Norris	NU
KZN	Palm Beach	Ptilophora diversifolia	NAT 3382	Norris	NU
KZN	Southern KZN (Drège, 1843)	Ptilophora spissa	33301	Suhr	LD
KZN	Southern KZN (Drège, 1843)	Ptilophora spissa	s.n.	Papenfuss	S
KZN	Southern KZN (Drège, 1843)	Ptilophora spissa	16292 / 4143	Papenfuss	W
EC	Gweqwe (Mkambati)	Ptilophora diversifolia	s.n.	E.G.J. Akhurst	NU
EC	Cape Morgan	Ptilophora diversifolia	35328	Flanagan	BOL
EC	Cape Morgan	Ptilophora spissa	7104	Pocock	GRA
EC	Algoa Bay, Cape Colony	Ptilophora diversifolia	SAM 102824	Norris	NU

Ptilophora helenae

KZN	Mission Rocks	Ptilophora helenae	KZN 2229	De Clerck & Leliaert	GENT/BOL
KZN	Mission Rocks (drift)	Ptilophora helenae	99071	Tronchin	BOL

KZN	First Rocks (drift)	Gelidium helenae	9769	Pocock	GRA
KZN	St. Lucia Rocks (drift)	Beckerella helenae	9772	Pocock	GRA
KZN	Richards Bay (drift)	Gelidium helenae	9633	Pocock	GRA
KZN	Richards Bay (drift)	Gelidium helenae	9631	Pocock	GRA
KZN	Richards Bay (drift)	Beckerella helenae	9633	Pocock	GRA
KZN	Richards Bay (drift)	Gelidium helenae	7103	Pocock	GRA
KZN	Richards Bay (drift)	Beckerella helenae	13241	Pocock	GRA
KZN	Richards Bay (drift)	Ptilophora pectinata	13241	Pocock	GRA

Ptilophora hildebrandtii

KZN	Tiger Reef	Ptilophora pectinata	KZN520	Bolton et al.	BOL
KZN	Sodwana Bay	Bekerella hildebrandtii	SAM100195 / NAT5285	Norris	NU

Ptilophora leliaertii

KZN	Uvongo (drift)	Beckerella sp. (nov.?)	10090	Pocock	GRA
KZN	Protea Banks, S. Pinnacle	Ptilophora hildebrandtii	99068	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora hildebrandtii	512	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora hildebrandtii	P1	Tronchin	BOL
KZN	Protea Banks	Ptilophora hildebrandtii	99069 / KZN881	Leliaert	BOL/GENT
KZN	Protea Banks, N. Edge	Ptilophora hildebrandtii	P9	Tronchin	BOL

Ptilophora pinnatifida

KZN	Park Rynie: Lander's Green	Ptilophora pinnatifida	009094 / NAT 2711	Norris	NU
KZN	Protea Banks, S. Pinnacle	Ptilophora pinnatifida	P6	Tronchin	BOL
KZN	Protea Banks, S. Pinnacle	Ptilophora pinnatifida	P7	Tronchin	BOL
KZN	Protea Banks, N. Edge	Ptilophora pinnatifida	P11	Tronchin	BOL
KZN	Protea Banks	Ptilophora pinnatifida	KZN882	Bolton	BOL/GENT
KZN	Protea Banks	Ptilophora pinnatifida	KZN896	Tronchin	BOL/GENT
KZN	Marina Beach	Ptilophora pinnatifida	NAT1342	Norris	WITS
KZN	Palm Beach	Ptilophora pinnatifida	KZN808	Tronchin	BOL/GENT
EC	Mkambati	Ptilophora pinnatifida	s.n.	E. G. J. Akhurst	NU
EC	Port Alfred/Kowie	Beckerella pinnatifida	7885	Norris	NU
EC	Port Alfred/Kowie	Ptilophora pinnatifida	s.n.	Becker	BOL
EC	Sharks Bay (Port Alfred)	Ptilophora pinnatifida	26030	Norris	NU
EC	Kleinemonde	Beckerella rumpii	6329	Pocock	GRA
EC	Algoa Bay, Cape Colony	Ptilophora pinnatifida	33300	Agardh	LD

Ptilophora rhodoptera

KZN	Park Rynie	Ptilophora rhodoptera	9522 / NAT370	Pienaar	NU
KZN	Protea Banks, Salmon Bank	Ptilophora rhodoptera	P12	Tronchin	BOL
KZN	Protea Banks, Salmon Bank	Ptilophora rhodoptera	KZN1991a	Tronchin	GENT

Ptilophora rumpii

KZN	Richards Bay (drift)	Ptilophora rumpii	7102	Pocock	GRA
KZN	Richards Bay (drift)	Ptilophora rumpii	13242	Pocock	GRA
KZN	Richards Bay (drift)	Rhodophylla rumpii	7102	Pocock	GRA
KZN	Richards Bay (drift)	Ptilophora rumpii	13242	Pocock	GRA
KZN	Richards Bay (drift)	Beckerella rumpii	9640	Pocock	GRA