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A re-evaluation of the genera *Beckerella* and *Ptilophora* (Gelidiales, Rhodophyta) based on molecular and morphological data

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Because the merger of the genera *Ptilophora* and *Beckerella* (Gelidiales, Rhodophyceae) into the single genus *Ptilophora* has not been universally accepted, we have reassessed this proposal using both molecular and morphological data. Phylogenetic analyses of DNA sequences for *rbc*L and large-subunit ribosomal DNA from 36 and 24 species of Gelidiales, respectively, place species of *Beckerella* and *Ptilophora* in a single, strongly supported clade. Morphological observations of vegetative structure in both genera confirm a morphological synapomorphy characterized by four concentric layers of different tissue types. Results of DNA sequence analyses indicate that *Beckerella* is paraphyletic with respect to *Ptilophora*, and constraining species of *Beckerella* and *Ptilophora* to form separate monophyletic clades required a large (10.2%) penalty to parsimony. Absence of surface proliferations has traditionally been the morphological character-state difference used to separate species of *Beckerella* from *Ptilophora*, but we have found such proliferations in four species of *Beckerella*, including the generitype *B. pinnatifida*. These results support the congeneric status of *Beckerella* with the earlier-described *Ptilophora*.

INTRODUCTION

Holmes (1896) proposed that a separate section of the genus *Ptilophora* Kützing (Gelidiales, Rhodophyta) should be created to contain species characterized by flattened fronds without proliferations on the surface, although he did not describe a new taxon. Later, 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 distinction 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 or absence of proliferations to be a good generic character and returned *P. 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 (1987) merged it with *Beckerella* after discovering surface proliferations in the type species of *Beckerella*, *B. pinnatifida* (J. Agardh) Kylin. Norris (1987) regarded the four-layered vegetative construction of *Ptilophora* (including *Beckerella*) species, which 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) as the major genus-defining feature, rather than proliferations, which he regarded as being probably a facultative response to epiphytic sponges.

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

Norris's (1987) 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). We have now reassessed the supposed diagnostic characters of *Beckerella* and *Ptilophora*, on the basis of a combination of 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* before its merger with *Beckerella*. We have also provided photomicrographs illustrating the characteristic four-layered vegetative construction in five additional species.

MATERIAL AND METHODS

Molecular analyses

Specimens of fresh material used for *rbc*L and large-subunit (LSU) ribosomal DNA sequence analyses were collected from

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field sites and dried in silica gel desiccant (Chase & Hills 1991). Vouchers for these specimens are located in the silica gel–dried algal collection at the Center for Marine Sciences, University of North Carolina–Wilmington (UNCW), Wilmington, NC, USA. Small pieces of dried herbarium specimens were used for a limited number of taxa for which field collections were not available. GenBank accession numbers for sequences generated from these specimens and specimen collection locations are listed in Table 1. Total genomic DNA was extracted following the protocols of either Freshwater & Rueness (1994) or Hughey *et al.* (2001). Amplifications of the chloroplast-encoded *rbc*L and a portion of the nuclear-encoded LSU were done as described in Thomas & Freshwater (2001). Sequencing reactions were performed using the Big Dye sequencing kit and protocol (Applied Biosystems, Foster City, CA, USA) and were analysed on either ABI Prizm 377 or 3100 Genetic Analyzer (DNA Analysis Facility, Center for Marine Science, UNCW). 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, Ann Arbor, MI, USA) and MacClade version 4.0 (W.P. Maddison & D.R. Maddison 2000). Characteristics of the aligned sequence data were determined and phylogenetic analyses were performed using MacClade and PAUP version 4.0b8 (Swofford 2001).

Two different molecular data sets were analysed in this study. The *rbc*L data set included sequences from 36 taxa. Because of 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 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.* 2002). Phylogenetic trees were generated from these data files using the maximum parsimony (MP), distance, and maximum likelihood (ML) methods.

MP analyses of the *rbc*L data set consisted of a two-part heuristic search. Initially, 1000 random sequence additions using MULTREES, STEEPEST DESCENT, and the nearest-neighbour–interchange branch-swapping algorithm (but keeping only 40 trees for each step) were performed. Trees found in this initial search were then swapped to completion using MULTREES, STEEPEST DESCENT, and the 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 100 random sequence additions, with MULTREES and TBR (*rbc*L), or 1000 replications of branch-and-bound searches (LSU). Distance trees were generated using neighbour-joining (NJ) 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 NJ 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 350 (*rbc*L) or 500 (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 those in the likelihood searches, were performed for both the *rbc*L and LSU sequence data files.

A combined *rbc*L + LSU data file, including the 10 analysed *Ptilophora* and *Beckerella* species, together with *Capreolia implexa* Guiry & Womersley as an outgroup, was used for constrained analyses. Branch-and-bound parsimony searches were performed with and without constraining of the resulting tree to include separate monophyletic clades for *Ptilophora* and *Beckerella* species.

Morphological observations

The occurrence and external morphology of surface proliferations was investigated (Table 1) using a Wild stereo dissecting photomicroscope. Comparisons were also made of the internal structure of surface proliferations in *P. diversifolia* (Suhr) Papenfuss, *P. prolifera*, *B. scalaramosa* Kraft, and *B. hildebrandtii* (Hauck) Kylin, by cutting transverse and longitudinal sections of their apical and basal portions.

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 1). Changes in vegetative structure with thallus age were investigated by cutting transverse sections in apical (within 2 mm of the branch apex), subapical (*c.* 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*, *B. 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 Axiocam compound photomicroscope.

RESULTS

Molecular analyses

The *rbc*L data set analysed included 36 species and 1400 nucleotide sites. Five hundred sixteen sites (36.9%) were variable and 408 (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 1636 steps (Fig. 1). Trees derived from distance, ML, 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 MP and ML trees and varied only in the position

Table 1. Species, collection locality, and GenBank accession numbers for taxa included in morphological and molecular analyses.

Species	Collection location	Morphology[1]	Accession number	
			*rbc*L	LSU
Beckerella				
B. hildebrandtii	Mombassa, Kenya (holotype)	Y	—	—
B. hildebrandtii	Tiger Reef, Bhanga Neck, KwaZulu-Natal, South Africa	—	AF522359	AF521178
B. mediterranea	Cape Matapan, Southern Peloponnesus, Greece (isotype)	Y	AF522360	AF521179
B. pectinata	Maroubra Bay, New South Wales, Australia (holotype)	Y	—	—
B. pinnatifida	Protea Banks, KwaZulu-Natal, South Africa	Y	—	—
B. pinnatifida	Sharks Bay, Port Alfred, Eastern Cape, South Africa	—	AF522361	AF521180
B. pterocladioides	Mokala, Madagascar (holotype)	Y	AF522362	AF521181
B. rumpii	Richards Bay, KwaZulu-Natal, South Africa (holotype)	Y	—	—
B. scalaramosa	Bulusan, Luzon, Philippines	Y	AF305804	AF296512
B. subcostata	Chiba, Japan	Y	—	—
B. subcostata	Fujisawa, Kanagawa, Japan	—	U16835	AF039546
Beckerella sp.[2]	Protea Banks and Palm Beach, KwaZulu-Natal, South Africa	Y	U16834	AF039547
Ptilophora				
P. diversifolia	Protea Banks, KwaZulu-Natal, South Africa	Y	AF305803	AF521182
P. prolifera	Cawaramup Bay, Western Australia, Australia	Y	—	—
P. rhodoptera	Protea Banks, KwaZulu-Natal, South Africa	Y	AF522365	AF521183
P. spissa	'Omsamculo', S. KwaZulu-Natal, South Africa	Y	—	—
Ptilophora sp.	Protea Banks, KwaZulu-Natal, South Africa	Y	AF522366	AF521184
Acanthopeltis				
A. japonica	Chikura-Town, Chiba, Japan	Y	—	—
Capreolia				
C. implexa	Port Philip Bay, Victoria, Australia	Y	L22456	AF039545
Gelidium				
G. asperum	Warnambool, Victoria, Australia	Y	—	—
G. abbottiorum	Breezy Point, Eastern Cape, South Africa	Y	—	—
G. canariensis	Puerto de la Cruz, Tenerife, Canary Islands	—	L22460	—
G. capense	Storm's River Mouth, Eastern Cape, South Africa	Y	—	—
G. capense	False Bay, Cape Peninsula, South Africa	—	L22461	—
G. caulacantheum	Porirua Harbor, North Island, New Zealand	Y	U00103	AF039544
G. chilense	Tongoy Bay, Coquimbo, Chile	Y	—	—
G. coulteri	Pacific Grove, California, USA	Y	—	—
G. coulteri	Balboa Peninsula, Orange Country, California, USA	—	U00105	—
G. crinale	Masonboro Inlet, North Carolina, USA	—	U00981	AF039543
G. divaricatum	Tokawa, Choshi, Chiba, Japan	—	U16828	—
G. floridanum	Sebastian Inlet, Florida, USA	—	U00107	AF039537
G. floridanum	Praia de Peruibe, Estado de Sao Paulo, Brazil	Y	—	—
G. japonicum	Cyoshi-City, Chiba, Japan	Y	—	—
G. japonicum	Keelung, Taiwan	—	AF501287	AF521185
G. latifolium	Portstewart, Co, Londonderry, Northern Ireland	Y	—	—
G. latifolium	Plouguerneau, Brittany, France	—	U00112	AF039540
G. microdonticum	Cahuita, Limon, Costa Rica	—	AF305799	—
G. micropterum	Clovelly, False Bay, Cape Peninsula, South Africa	Y	—	—
G. micropterum	Kommetjie, Cape Peninsula, South Africa	—	U00446	—
G. pacificum	Matsugahana, Amatsukominato, Chiba, Japan	—	U16832	—
G. pluma	Hilo, Hawai'i, Hawaiian Islands	—	AF522367	—
G. pristoides	Clovelly, False Bay, Cape Peninsula, South Africa	Y	—	—
G. pristoides	False Bay, Cape Peninsula, South Africa	—	U01044	AF039541
G. pteridifolium	Isipingo, Kwazulu-Natal, South Africa	Y	—	—
G. pulchellum	Fanore, Co, Clare, Ireland	Y	—	—
G. pusillum	Ambletusa, France	Y	—	—
G. pusillum	Cancale, Brittany, France	—	U01000	—
G. pusillum	Fedje, Norway	—	U00999	AF039542
G. rex	Tongoy Bay, Coquimbo, Chile	—	AF305801	—
G. sesquipedale	Biarritz, France	Y	—	—
G. sesquipedale	Aramar, Asturias, Spain	—	L22071	AF039539
G. serrulatum	Mochimo, Venezuela	—	U01042	AF039538
G. vagum	Iwate, Japan (Shimada *et al.* 1999)	—	AB017680	—
G. vittatum	Oudekraal, Cape Town, South Africa	Y	—	—
Gelidium sp.	Piha, North Island, New Zealand	—	U01043	—
Pterocladia				
P. lucida	Owhiro Bay, South Wellington, New Zealand	Y	U01048	AF039550

Table 1. Continued.

Species	Collection location	Morphology[1]	Accession number *rbc*L	Accession number LSU
Pterocladiella				
P. bartlettii	Cahuita, Limon, Costa Rica	Y	—	—
P. bartlettii	Port Aransas, Texas, USA	—	AF305807	AF296515
P. caerulescens	Sandy Beach, Oahu, Hawaiian Islands	Y	—	—
P. capillacea	Cottesloe Reef, Perth, Western Australia, Australia	Y	—	—
P. capillacea	Torre a Mare, Bari, Italy	—	U01888	AF308797
P. melanoidea	Mallorca, Spain	—	U01046	AF039548

[1] Y = specimens were used for morphology.
[2] This specimen was misidentified when collected and was originally published as *Ptilophora pinnatifida* (Freshwater *et al.* 1995).

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 at more than two sites. Because of the small size of the indels, sites coded as gaps were treated as a fifth base in parsimony analyses. The LSU alignment included 138 variable sites (11.9%), of which 97 (8.4%) 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 three minimal trees of 214 steps (Fig. 2). Tree topologies resulting from distance, ML, and quartet-puzzling analyses did not differ significantly from the MP 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.

MP analysis of the combined *rbc*L + LSU data file for *Ptilophora* and *Beckerella* species resulted in a single minimal tree of 255 steps. 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 281 steps. This is a penalty to parsimony of 26 steps, which is 10.2% of the unconstrained parsimony tree length.

Morphological observations

Nine of the 14 species of *Ptilophora* and *Beckerella* examined were found to have surface proliferations [viz. *P. spissa* (Suhr) Kützing, *P. diversifolia* (Fig. 3), *P. rhodoptera* Norris, *P. prolifera*, *Ptilophora* sp., *B. pinnatifida* (Fig. 4), *B. hildebrandtii* (Fig. 5), *Beckerella* sp., and *B. scalaramosa*]. Three species, *P. 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. 3) and *P. prolifera* produced proliferations like those described in Norris (1987) 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 (not illustrated), although these were relatively few in number (three in total on a 4-cm-long frond for example). Similarly, proliferations occurring on *B. pinnatifida* (Fig. 4) and *B. hildebrandtii* (Fig. 5) were rare, although many surface proliferations have been reported on sponge-covered specimens of *B. pinnatifida* (Norris 1987). Surface proliferations on *B. pinnatifida* were mostly cylindrical, whereas those on *B. hildebrandtii* were ligulate or sometimes pinnate. Only one of eight specimens of *Beckerella* sp. was found to produce surface proliferations, consisting of two compressed and pinnately branched enations. Two types of proliferations were found on *B. scalaramosa* (not illustrated): those produced distally on the thallus and those in proximal regions. The former, observed on one specimen, were subcylindrical to cylindrical, sometimes branched, 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 branch bearing them because they had a single-layer outer cortex. The latter were minute (*c.* 550 μm long, *c.* 150 μm in diameter at their base), were produced only 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 in species of both *Ptilophora* and *Beckerella* (Fig. 6). Short proliferations (≤ 1 mm in length) consisted almost entirely of cortical cells and lacked medullary filaments, as did apical parts of longer proliferations.

In longitudinal and transverse sections of second-order branches, all the species of *Ptilophora* and *Beckerella* studied had a vegetative structure comprising four concentric tissue layers (Figs 7–11). Structural details of the tissue layers are included in the descriptions of these species (e.g. Kraft 1976; Norris 1987). The original descriptions and illustrations of *B. irregularis* Akatsuka & Masaki (1983) and *B. biserrata* Børgesen (1943) were referred to because no material of these two species was available for sectioning and both had a vegetative structure that agreed with the pattern present in the species studied.

The distinctness of the four tissue layers varied with the age of the thallus section examined. In apical transverse sections of *P. prolifera*, *B. subcostata*, *B. pectinata*, and *B. scalaramosa*, the outer cortex was one cell layer thick. The cortical filaments were disorganized 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 the inner cortex was distinct. The inner cortex comprised most of the vegetative structure and consisted

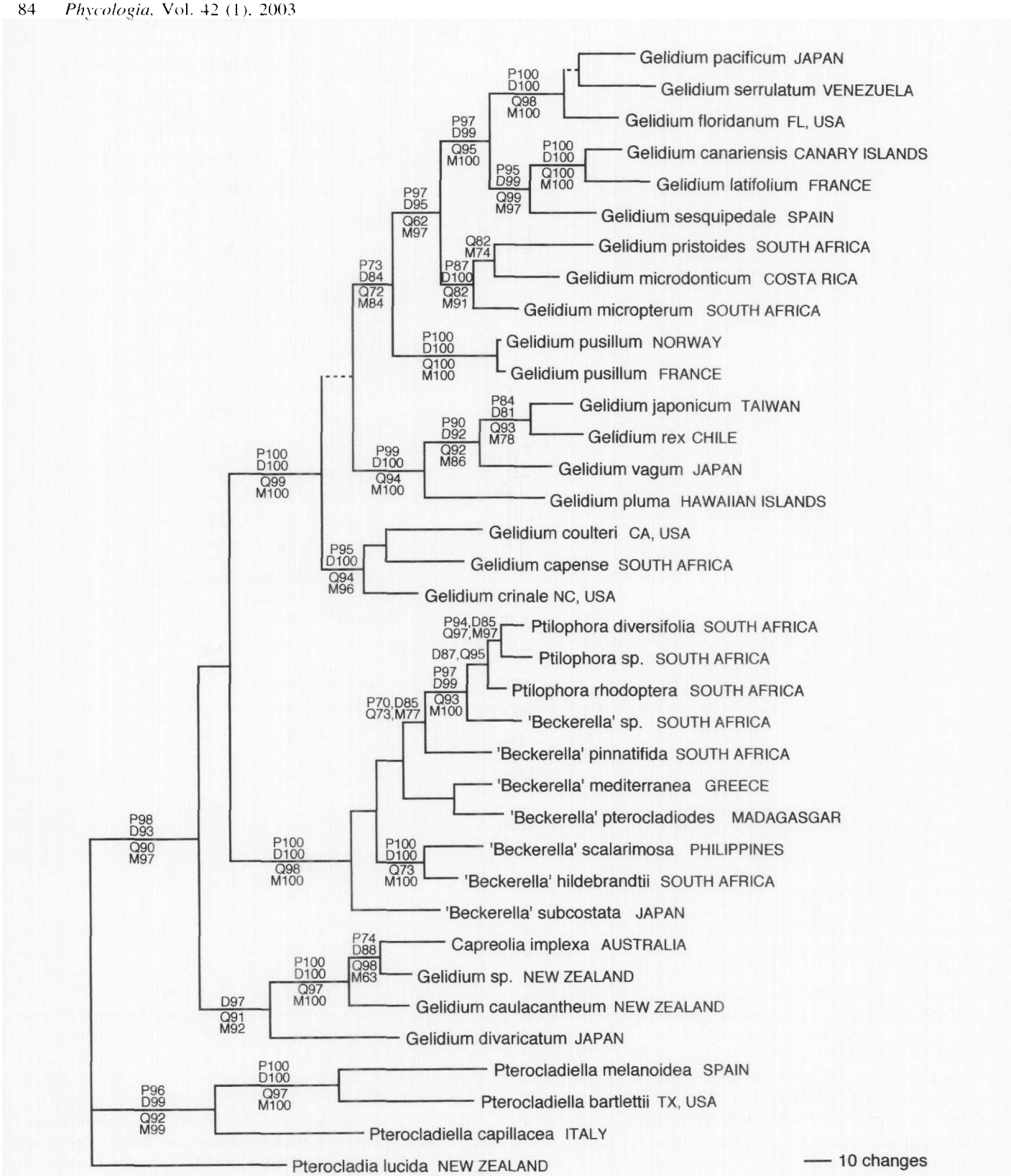

Fig. 1. One of three minimal trees [length (L) = 1636; consistency index (CI) = 0.396] resulting from MP searches of *rbc*L sequences from 36 species of Gelidiales. Branches not present in all minimal trees are represented by dashed lines. Bootstrap support (%) and quartet-puzzling reliability values are given for branches when greater than 70 (P, parsimony; D, distance; Q, quartet puzzling; M, maximum likelihood).

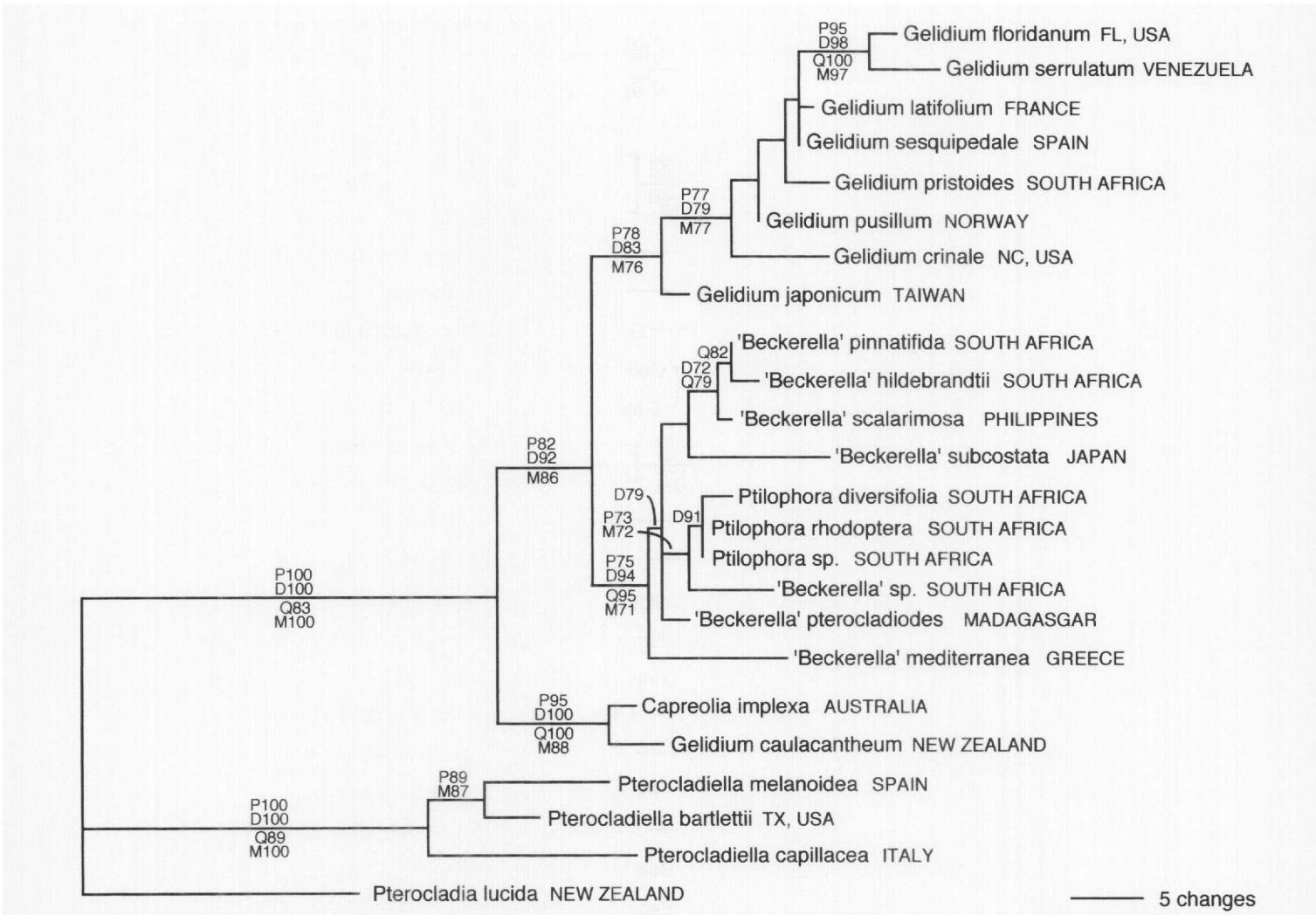

Fig. 2. One of three minimal trees [length (L) = 214; consistency index (CI) = 0.661] resulting from MP searches of partial LSU sequences (1159 sites) from 24 species of Gelidiales. Bootstrap support (%) and quartet-puzzling reliability values are given for branches when greater than 70 (P, parsimony; D, distance; Q, quartet puzzling; M, maximum likelihood).

of variably sized ovoid to elliptical cells that were bigger and thicker-walled than the outer cortical cells. The medulla was indistinct apically. Rhizines were only found scattered throughout the core in *B. scalaramosa* and *P. 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 were 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 transversely 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 the main axes, which consist instead of homogenously distributed pigmented cortical cells in a network of transversely and longitudinally oriented rhizines.

None of the members of the Gelidiaceae examined, apart from *Ptilophora* and *Beckerella*, had the characteristic vegetative structure described above because 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 observed only in *G. 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 *G. vittatum* (Linnaeus) Kützing, rhizines were concentrated mostly in the medulla. The vegetative structure of *P. lucida*, *G. coulteri* Harvey, *G. chilense* (Montagne) Santelices & Montalva, *G. pulchellum* (Turner) Kützing, *G. latifolium* Bornet *ex* Hauck, *G. caulacantheum* J.

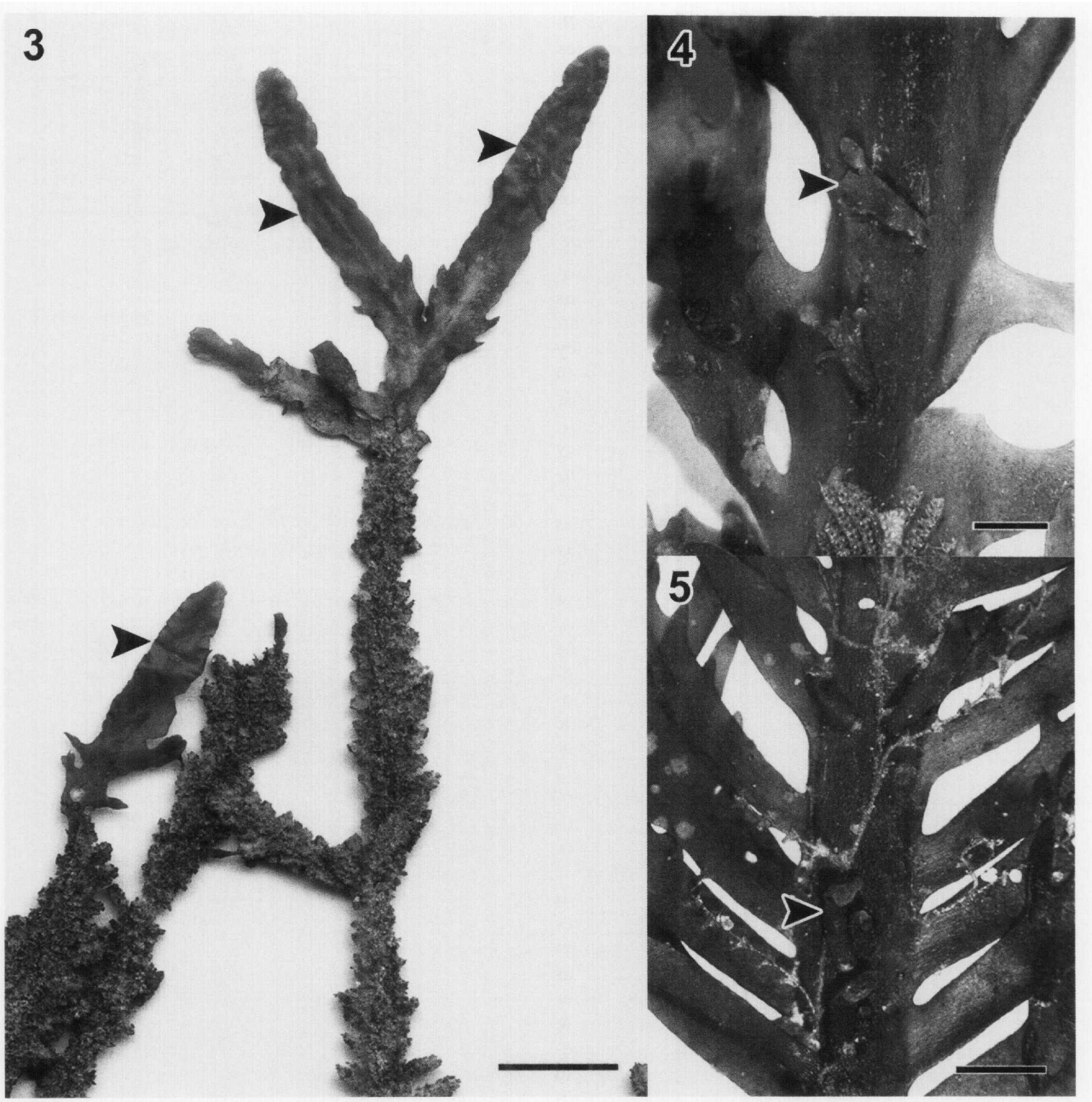

Fig. 3. *Ptilophora diversifolia.* Branches covered in a sponge epiphyte. Only the distal fronds have smooth surfaces (arrowheads) free from surface proliferations and sponge. Scale = 1 cm.
Fig. 4. *Beckerella pinnatifida.* A branch with two surface proliferations (arrowhead) issuing from the midrib. Scale = 1 mm.
Fig. 5. *Beckerella hildebrandtii.* A main axis with surface proliferations (arrowhead) along its length. Scale = 1 mm.

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 were 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 in 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 vary among those species that possess 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*, *B. 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. Because of the length and single-layered outer cortex of the distally occurring surface proliferations of *B. scalaramosa*, these proliferations 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, because they are produced only at the bases 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 differences noted in surface proliferation morphology and arrangement are probably species specific. The within-species variation observed 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. On the basis of these molecular data and the inconsistency found in the key morphological character distinguishing *Ptilophora* and *Beckerella*, we regard their maintenance as separate genera as untenable.

Although *Ptilophora* and *Beckerella* were not resolved as separate monophyletic clades in molecular analyses, species of both genera were 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 included 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 on the tree-building method, a moderately to strongly supported clade in the LSU tree.

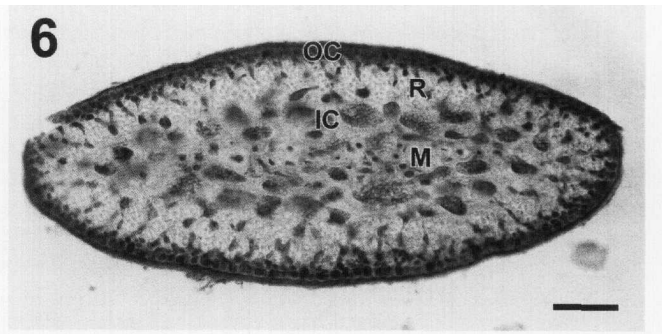

Fig. 6. *Beckerella hildebrandtii*. Transverse section through the base of a small surface proliferation. OC, outer cortex; R, rhizine layer; IC, inner cortex; M, medulla. Scale = 50 μm.

Morphological comparisons of species belonging to the Gelidiales show that only species of *Ptilophora* and *Beckerella* have a vegetative structure characterized by four distinct, concentric layers of homogeneous tissue types. The presence of an inner cortex of inflated cells is especially characteristic of these two genera. Species differ predominantly in the thickness of the rhizine band and inner cortex and the size of the inner cortical cells. We did not determine whether these differences were species specific or indicative of different thallus ages, or whether they reflected the environmental conditions experienced during growth.

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 [e.g. in *B. irregularis*, which lacks a midrib (Akatsuka & Masaki 1983)]. We suggest that transverse sections of second-order branches be made to confirm generic identification, because the anatomy of the oldest parts of the thalli are complicated by increased cortication.

The results of this study support the conclusion drawn by Norris (1987) that *Ptilophora* and *Beckerella* are congeneric. The species of *Beckerella* should be referred to *Ptilophora*, which therefore now contains 14 species (Table 2). Norris's (1987) emendation of *Ptilophora* was concise and comprehensive and does not need to be repeated here, except that we would note an increase in the stated maximum size of thalli

Table 2. List of species currently included in *Ptilophora*, with corresponding nomenclatural authorities.

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

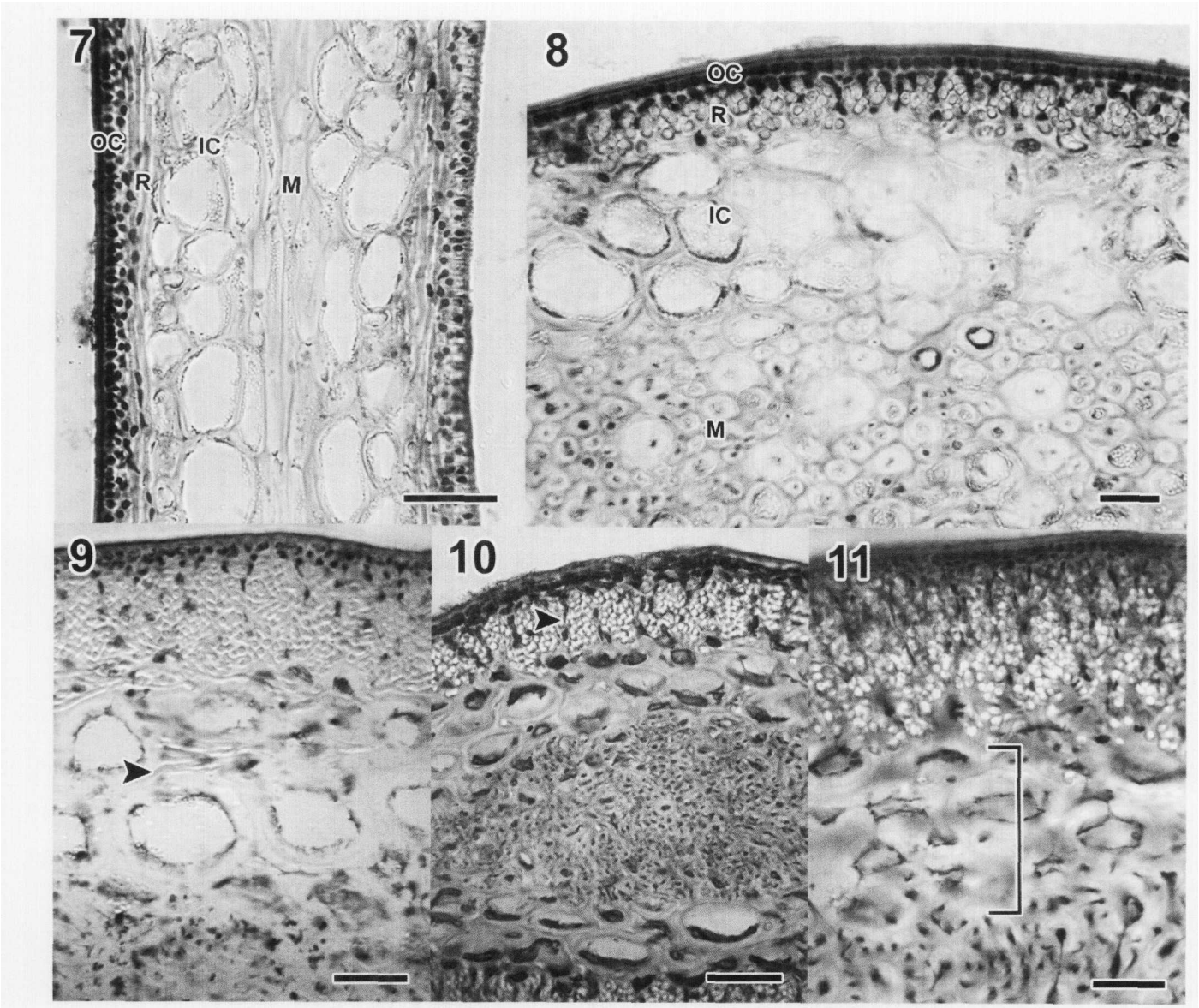

Fig. 7. *Beckerella subcostata.* Longitudinal section of second-order branch. OC, outer cortex; R, rhizine layer; IC, inner cortex; M, medulla. Scale = 50 μm.
Fig. 8. *Beckerella subcostata.* Transverse section of second-order branch. OC, outer cortex; R, rhizine layer; IC, inner cortex; M, medulla. Scale = 25 μm.
Fig. 9. *Beckerella scalaramosa.* Transverse section of second-order branch with transversely oriented rhizines (arrowhead) in the inner cortex. Scale = 50 μm.
Fig. 10. *Beckerella pinnatifida.* Transverse section of second-order branch with anticlinal filaments of pigmented cortical cells (arrowhead) traversing the rhizine layer. Scale = 50 μm.
Fig. 11. *Ptilophora diversifolia.* Transverse section of second-order branch with an inner cortex four cell layers thick (bracketed). Scale = 50 μm.

from 0.5 to 1 m, on the basis of observations of *P. subcostata* (Okamura 1909). Also, the generitype *P. spissa* is only known from the type specimen, collected over a century ago in the southern KwaZulu-Natal Province, South Africa. It has not been subsequently collected, despite the recent collecting efforts of the authors and the previous research conducted by Norris (1987, 1992). Preliminary observations in a current study of South African *Ptilophora* species indicate that the *P. spissa* specimen may be an aberrant morphology of another species from the same locality.

Our continuing studies of *Ptilophora* are now addressing questions of species relationships within the genus. This includes describing two newly discovered species, reassessing the status of all currently recognized species, and developing a species key. The recent mergers of *Beckerella* with *Ptilophora* (Norris 1987), *Yatabella* Okamura with *Acanthopeltis* Okamura *in* Yatabe (Shimada *et al.* 1999), and *Onikusa* Akatsuka and *Suhria* J. Agardh *in* Endlicher with *Gelidium* (Tronchin *et al.* 2002) leave eight recognised genera within the Gelidiales. Further study of *Acanthopeltis*, *Capreolia* Guiry & Womersley, and *Porphyroglossum* Kützing is necessary to determine the status of these genera within a phylogeny-based natural classification of the Gelidiales.

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