

# *Ptilophora leliaertii* and *Ptilophora coppejansii*, two new species of Gelidiales (Rhodophyta) from South Africa

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*Ptilophora leliaertii* Tronchin et De Clerck sp. nov. and *Ptilophora coppejansii* Tronchin et De Clerck sp. nov. are described from Protea Banks reef situated off the southern coast of the KwaZulu-Natal Province in South Africa. They have thus far been collected only on this reef system. *Ptilophora leliaertii* is distinguishable by its compound pinnate branching and the concave nature of its flattened branches. *Ptilophora coppejansii* is distinguishable by short, closely spaced pinnae in its distal parts, arising from irregularly arranged indeterminate branches with broad flexuous alae and a distinct midrib. Analyses of *rbcL* and LSU gene sequences resolve both species as distinct, well-supported taxa, with *P. coppejansii* sister to *Ptilophora diversifolia* (Suhr) Papenfuss, and *P. leliaertii* sister to *Ptilophora helenae* (Dickinson) R. Norris. The reinstatement of *P. helenae*, a species recently placed in synonymy with *Ptilophora pectinata* (A. Gepp & E. Gepp) R. Norris from Australia, is proposed here based on molecular, morphological and biogeographical evidence.

**Key words:** Gelidiales, LSU, Protea Banks, *Ptilophora*, *rbcL*, Rhodophyta, South Africa, taxonomy

## Introduction

*Ptilophora* Kützinger, a red algal genus distributed in the Indo-West Pacific, is known predominantly from subtidal habitats down to more than 100 m below m.s.l. Consequently, the genus is seldom encountered, and three of the 14 currently recognized species have been collected only from drift. *Ptilophora* species are predominantly temperate (only two species known from tropical waters) and biogeographically distinct with restricted range distributions.

The genus was erected by Kützinger (1847) who emphasised the presence of surface proliferations and a four-layered vegetative structure in his description. Subsequently, phycologists disagreed on the importance of surface proliferations in defining *Ptilophora*. J. Agardh's (1885) description of *Ptilophora pinnatifida* J. Agardh made specific mention of its lack of surface proliferations. Schmitz (1894) regarded proliferations as not being useful as a generic character. He returned *Ptilophora prolifera* (Harvey) J. Agardh and *P. pinnatifida* to the genus *Gelidium* Lamouroux, since they lacked the hyaline

bristles (later established to be sponge spicules by Papenfuss, 1940) found on *Ptilophora diversifolia* (Suhr) Papenfuss and *Ptilophora spissa* (Suhr) Kützinger, which Schmitz believed was the diagnostic character for *Ptilophora*. *Gelidium proliferum* Harvey and *Gelidium pinnatifidum* (J. Agardh) Schmitz were later reincorporated into *Ptilophora* (Papenfuss, 1940; Norris, 1987). Schmitz & Hauptfleisch (1897) and Papenfuss (1940) continued to attach major importance to surface proliferations as a diagnostic character for the genus. Kylin (1956) delimited a new genus *Beckerella* Kylin into which he placed some species of *Ptilophora* and *Gelidium* that lacked surface proliferations and had a less-distinct midrib in their distal parts. Norris (1987) merged *Beckerella* into *Ptilophora* following his observation of surface proliferations in the type species, *Beckerella pinnatifida* (J. Agardh) Kylin, a decision that was not generally accepted (e.g. Barreto *et al.*, 1997; Kraft *et al.*, 1999; Murase *et al.*, 1989; Silva *et al.*, 1996; Trono, 1997). Tronchin *et al.* (2003) later supported this merger with new molecular and morphological data.

The concept of *Ptilophora* is clearly defined in Norris (1987). In summary, *Ptilophora* is characterized by a large distichously branched frond (sometimes bearing minute surface proliferations).

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It usually has a distinct midrib in its 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 species descriptions, they have not been used as diagnostic characters for any of the species.

Recent taxonomic research on *Ptilophora* (Norris, 1987, 1990, 1992) 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 known and described, and most have been repeatedly collected. This previous research greatly facilitated the identification of two new species endemic to this area, namely *Ptilophora coppejansii* Tronchin et De Clerck and *Ptilophora leliaertii* Tronchin et De Clerck, 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 robust molecular support from DNA sequence data. This is the first time that new species in the genus *Ptilophora* have been proposed with the support of molecular data. *Ptilophora leliaertii* has previously been misidentified as *Ptilophora pinna-tifida* (Freshwater *et al.*, 1995; Bailey & Freshwater, 1997; Freshwater & Bailey, 1998; Freshwater *et al.*, 1999; Thomas & Freshwater, 2001; Rico *et al.*,

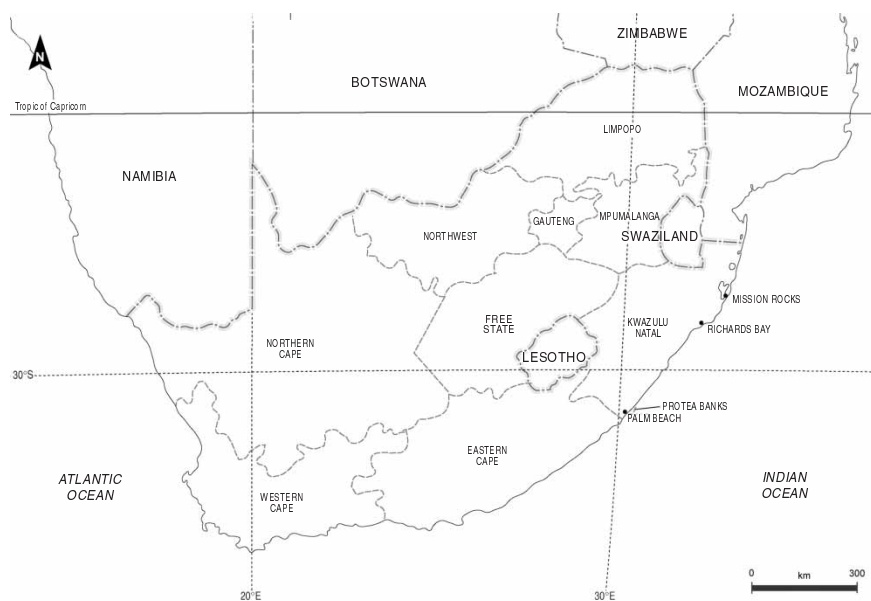
2002) and referred to as *Beckerella* sp. by Tronchin *et al.* (2003) who also referred to *Ptilophora coppejansii* as *Ptilophora* sp.

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

## Materials and methods

### Morphological observations

Specimens of the new species were obtained in the course of SCUBA diving on Protea Banks reef (Fig. 1) during August 1999, January and June 2000 and February 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 type specimens of thirteen of the fourteen species). Observations of whole specimens were made using a Wild M10 stereo dissecting photomicroscope and photographs were taken with an Olympus Camedia digital camera. Hand sections were cut from pressed or silica-dried specimens, stained with 1% aniline blue and preserved in a 50% Karo<sup>TM</sup> solution. Using image analysis software (UTHSCSA Image tool v. 3, Univ. Texas Health Science Center, San Antonio, TX, USA), morphometric data were obtained from various specimens of *P. leliaertii*, *P. 'pectinata'* from South Africa (formerly *P. helenae*) and *P. pectinata* from Australia, including the type specimens of the three species.



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

### Molecular analyses

Specimens used for gene sequence analyses were collected from field sites and dried using silica gel desiccant. Samples were also taken from herbarium specimens, including type material. Sequences generated from these specimens were uploaded to the GenBank database and given accession numbers (Table 1). Total genomic DNA was extracted following the protocols of Freshwater & Rueness (1994) or Hughey *et al.* (2001). Amplifications of the chloroplast-encoded *rbcL* gene and a portion of the nuclear-encoded large-subunit ribosomal RNA gene (LSU) were as described in Thomas & Freshwater (2001). Sequencing reactions performed using the Big Dye sequencing kit and protocol (Applied Biosystems, Foster City, CA, USA) were analysed on either an ABI Prizm 377 or a 3100 Genetic Analyzer (DNA Analysis Facility, Center for Marine Science, UNCW). The sequences of primers used in this study are presented in 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).

Distance, maximum parsimony and maximum likelihood analyses were performed on *rbcL* and LSU data files using PAUP (v. 4.0b10, Swofford, 2001). Eighteen specimens were included in analyses of the *rbcL* sequence data, and 13 specimens in analyses of the partial LSU sequence data. Due to missing data at the 5' ends of *rbcL* and LSU sequences, the first 67 sites of the 1467 bp *rbcL* gene and first seven 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 *rbcL* 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 & 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 *rbcL* 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 was used. Optimality criteria for maximum likelihood analyses were determined with Modeltest v. 3.06 (Posada & Crandall, 1998). Maximum likelihood analyses of *rbcL* 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 (*rbcL*) 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 et De Clerck sp. nov.

### Diagnosis

Plantae usque ad 35 cm altae, crescentes ex haptero fibroso e partibus robustis teretibus constanti.

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

Species	Collection location	Accession Number	
		<i>rbcL</i>	LSU
<i>P. copejansii</i>	Protea Banks, KwaZulu-Natal, South Africa	AF522366	AF521184
<i>P. diversifolia</i>	Protea Banks, KwaZulu-Natal, South Africa	AF522364	AF521182
<i>P. hildebrandtii</i>	Tiger Reef, Bhanga Neck, KwaZulu-Natal, South Africa	AF522359	AF521178
<i>P. mediterranea</i>	Cape Matapan, S. Peloponnesus, Greece (isotype)	AF522360	AF521179
<i>P. leliaertii</i>	Palm Beach, KwaZulu-Natal, South Africa	U16834	AF039547
	Protea Banks, KwaZulu-Natal, South Africa	AY344047	—
<i>P. pinatifida</i>	Sharks Bay, Port Alfred, Eastern Cape, South Africa	AF522361	AF521180
	Protea Banks, KwaZulu-Natal, South Africa	AY344046	—
<i>P. pectinata</i>	Bongin Bangin, New South Wales, Australia	AY344044	AY345881
<i>P. 'pectinata'</i>	Mission Rocks, KwaZulu Natal Province, South Africa	AY344045	AY345880
<i>P. pterocladoides</i>	Mokala, Madagascar (holotype)	AF522362	AF521181
<i>P. rhodoptera</i>	Protea Banks, KwaZulu-Natal, South Africa	AF522365	AF521183
<i>P. rumpii</i>	Richards Bay, KwaZulu-Natal, South Africa	AY345882	—
<i>P. scalaramosa</i>	Bulusan, Luzon, Philippines	AF305804	AF296512
<i>P. subcostata</i>	Fujisawa, Kanagawa, Japan	U16835	AF039546
<i>Capreolia implexa</i>	Port Philip Bay, Victoria, Australia	L22456	AF039545

Rhizoidea decumbentia ramosa in axe principali inferiore et ramis inferioribus interdum orientia. Rhizoidea cylindrica, ad apices haptera adligantia aspergilliformia producentia. Axis inferior cylindricus (ca. 2 mm in diametro), anguste alatus in ramis inferioribus, complanatus concavusque in segmentis pinnatis costa minus distincta. Ramificatio usque ad sexies disticha, regularis, pinnata composita, opposita ad alternata. Rami complanati plerumque 1.5–2 mm lati, satis aequae dispositi, distantes 1–2 mm a se, apicibus rotundatis vel angustatis in acumen, praesertim in pinnis juvenibus vel primordialibus. Rami concavi et (pinnis ultimis exceptis) plerumque ramis sustentibus latiores (latissimi parte 3/10 latitudinis totius), basibus ramum sustentem fere aequantibus. Proliferationes superficiales raras. Structura vegetativa 4-strata. Cellulae corticis exterioris rotundae ad quadrangulares, cingentes zonam distinctam rhizinarum parietibus crassis a seriebus anticlinalibus cellularum corticis exterioris perductam. Cortex interior 3–4-stratus, cellulis periclinalibus, rotundis ad ellipticis. Medulla filamentosa. Partes fertiles ad vel prope apices ramulorum lateralium portatae. Cystocarpia bilocularia, plerumque protuberantia distali instructa. Sori spermatangiales tetrasporangialesque elliptici, in ramulis brevibus parum tumidis portati. Tetrasporangiae cruciatim divisa.

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 less distinct midrib. Up to six orders of regular distichous, compound pinnate, opposite to alternate branches. 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 the ultimate pinnae, generally broader (up to 30%) than their bearing branch, and roughly as wide as the bearing branch at their base. 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 born terminally or subterminally on lateral branchlets. Cystocarps are bilocular and usually have a distal protuberance. Spermatangial and tetrasporangial sori elliptical, born on short, slightly swollen branchlets. Tetrasporangia cruciately divided.

**ETYMOLOGY:** This species is named after Frederik Leliaert of the Phycology Research Group at Ghent University, who collected the first attached specimen of the species, all previously collected specimens having come from beach-cast drift.

**HOLOTYPE:** 99068 (BOL) (Fig. 2), 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) and Palm Beach (12 km south 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 & Bolton; 29 vi 2000; KZN 1857 (GENT). Location unknown; Simons; 1966; 109599 (BOL). Protea Banks, Southern Pinnacle, – 34 m; Leliaert; 20 viii 1999; 512 (BOL) – wet preserved. Palm Beach (KZN) – drift; Hommersand; 23 vii 1993; s.n. (BOL). 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.E. 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).



Where the rhizoids contact the substratum they produce brush-like anchoring haptera composed of elongate aseptate cells (Fig. 4). 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 branchlets in the 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 the lower order branches. Branches are flattened and concave in the pinnate regions (Fig. 5) 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 (up to 2.5) mm broad and fairly uniformly spaced, generally 1–2 (up to 2.5) mm apart. Branch apices can taper to a sharp point with a protuberant apical cell (Fig. 6) particularly in young or primordial pinnae, and become rounded with age. Apices of indeterminate branches are often tridentate (Fig. 7).

In flattened regions, branches, except the 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. 8). 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. 9) 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. 10). 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)  $\mu\text{m}$  long, 5–8  $\mu\text{m}$  wide, with a length:width ratio of 1–2. Rhizines are 3–5  $\mu\text{m}$  in diameter and form a distinct band traversed by anticlinal rows of pigmented outer cortical cells. These cells are angular, and more elongated (length/width ratio is greater than 2). The inner cortex consists of three to four layers of periclinally arranged round to elliptical cells in transverse section, (27) 29–44 (51)  $\mu\text{m}$  long, (15) 20–28 (37)  $\mu\text{m}$  wide, with a length/width ratio of 0.8–2. The medulla consists of thick-walled filaments that are round in transverse section and 7–12  $\mu\text{m}$  in diameter, and is

devoid of rhizines. Larger cells can sometimes occur in the medulla, approximating the appearance of the small rounded inner cortical cells in transverse section. In higher order branches the medulla and midrib become less distinct. In proximal parts the stratified vegetative structure becomes obscured by increased cortication and rhizine concentration.

#### *Reproduction*

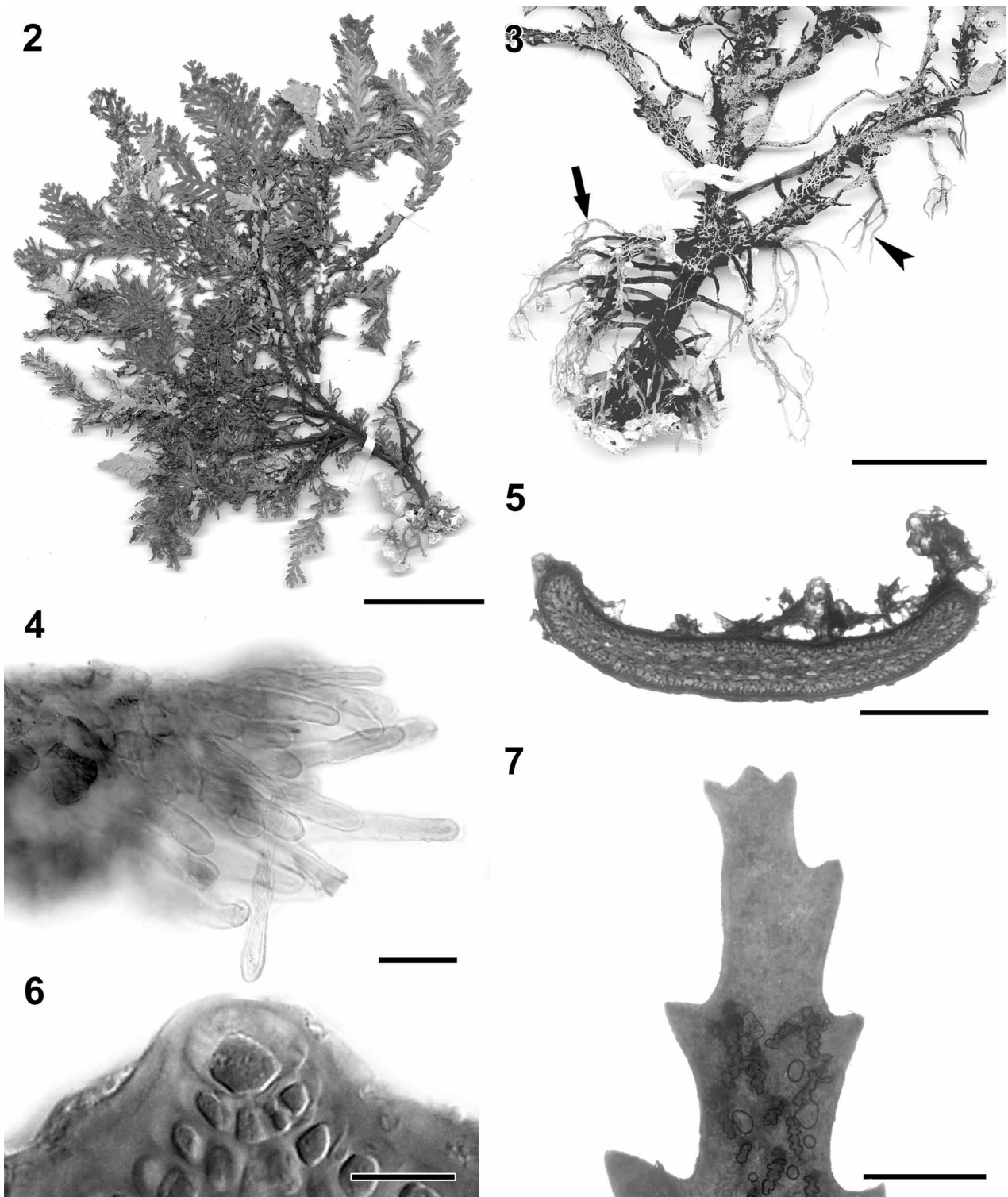
Reproductive structures are borne apically or subapically on lateral branchlets. Cystocarps are borne either on short branchlets or terminally on narrow ligules (up to 5 mm in length) usually with a distal protuberance. The cystocarps are bilocular, locules being elliptical in surface view, with one centrally placed ostiole that may have a peristome (Fig. 11) or may be depressed.

Spermatangia are borne on short, slightly lobed branchlets. They form a continuous single-layered outer cortex. Spermatangial sori develop on both surfaces of the pinnae and are surrounded by a sterile margin, which is too narrow to be apparent in surface view. Spermatangial initials can be easily recognized by their elongate shape and transverse division lines (Fig. 13).

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. 12). Sori are usually surrounded by a very thin sterile margin but may extend all the way around the flattened pinnule. The darkly pigmented tetrasporangia can usually be seen in surface view. Tetrasporangia are cruciately divided and when viewed in transverse sections are up to 58  $\mu\text{m}$  long and 40  $\mu\text{m}$  wide, with a length/width ratio of 1.2–2.4 (Fig. 14).

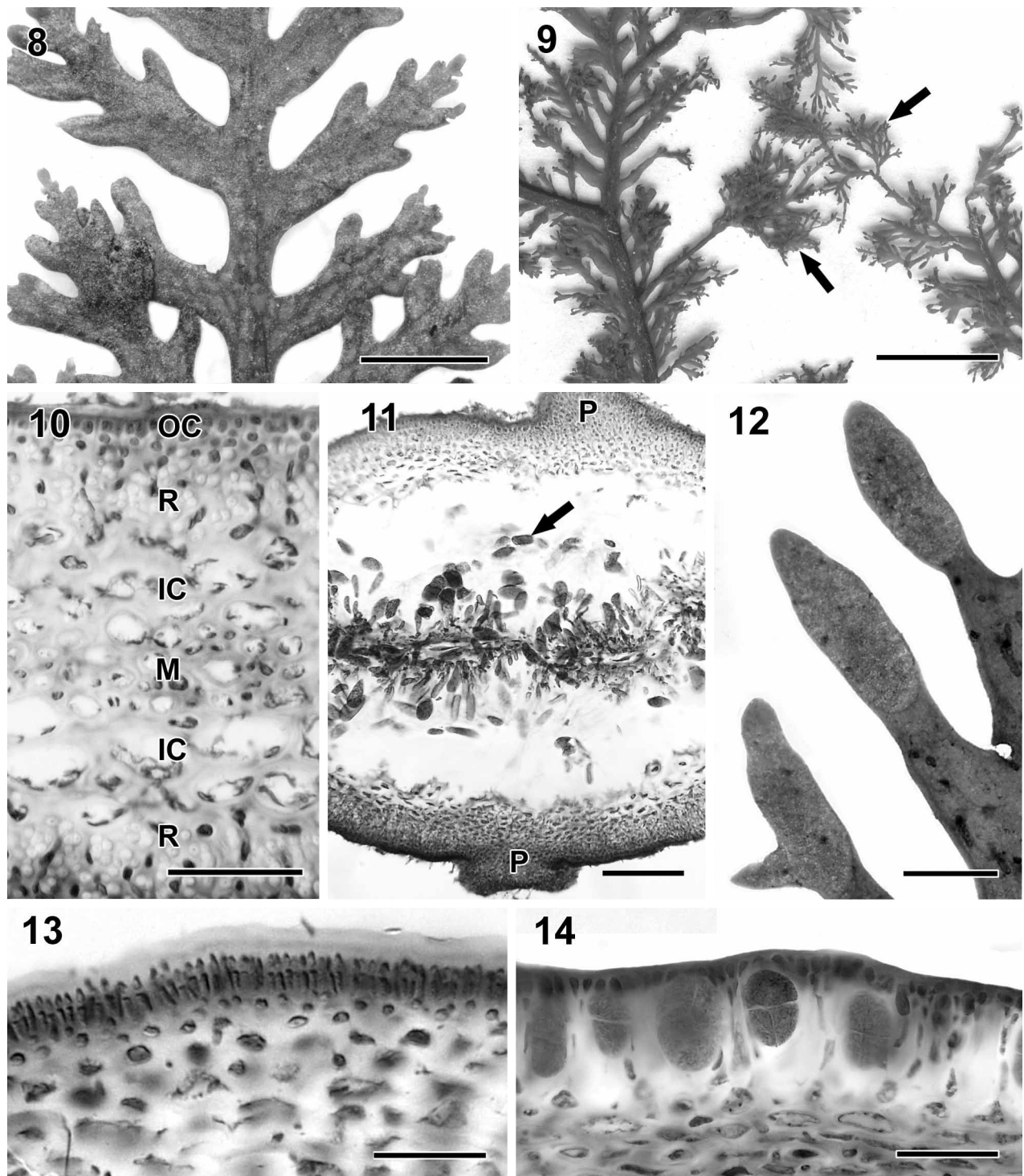
#### *Epiphytes*

*Acrosorium acrospermum* (J. Agardh) Kylin and a diversity of sponges, crustose corallines and hydroids are common epiphytes on *Ptilophora leliaertii*. Sponges and hydroids tend to attach to the concave surface of the frond (Fig. 5), while crustose corallines and *A. acrospermum* tend to attach to the convex surface. Sponges are often nestled within and fill the concavity, but may eventually envelop both surfaces of the branch. *Acrosorium acrospermum* is found predominantly on ultimate branches, its growth following the pattern of the underlying branches, and is only noticeable when it produces thin, flattened fertile lobes (with scattered, darkly-pigmented sori) that extend beyond the blade margins.



**Figs 2–7.** *Ptilophora leliaertii*. Fig. 2. Holotype specimen covered in places by sponge and crustose coralline epiphytes. Fig. 3. Holdfast with rhizoids developing from the lower axis (arrow) as well as from lower branches (arrowhead). Fig. 4. Brush-type anchoring hapteron of elongate aseptate cells developing from the end of a cylindrical rhizoid. Fig. 5. Transverse section of a distal concave branch. A hydroid and sponge epiphytes are attached predominantly to the concave surface of the frond. The sponge spicule skeleton was poorly preserved during the sectioning procedure. Fig. 6. Surface view of protuberant apical cell. Fig. 7. Surface view of apical tip of pinnate branch. Scale bars represent: Fig. 2, 4 cm; Fig. 3, 1 cm; Fig. 4, 50  $\mu\text{m}$ ; Fig. 5, 250  $\mu\text{m}$ ; Fig. 6, 10  $\mu\text{m}$ ; Fig. 7, 1 mm.





**Figs 8–14.** *Ptilophora leliaertii*. Fig. 8. View of convex surface of compound branch. Fig. 9. An aberrant morphology of this species where finely branched, lateral proliferations (arrows) are produced. Fig. 10. Transverse section of second order branch. Outer cortex (OC); rhizine band (R); inner cortex (IC); medulla (M). Fig. 11. Transverse section of biloculate cystocarp with mature carpospores (arrow) and ostioles with peristomes (P). Fig. 12. Surface view of three pinnae with lightly pigmented terminal tetrasporangial sori. The sorus of one of the pinnae extends into a lateral pinnule. Fig. 13. Transverse section of fertile region of pinnule with an outer cortex of spermatangial initials dividing transversely. Fig. 14. Transverse section of tetrasporangial branchlet with mature, cruciately divided tetrasporangia. Scale bars represent: Fig. 8, 3 mm; Fig. 9, 1 cm; Fig. 10, 50  $\mu$ m; Fig. 11, 100  $\mu$ m; Fig. 12, 1 mm; Fig. 13, 25  $\mu$ m; Fig. 14, 50  $\mu$ m.

*Ptilophora coppejansii* Tronchin et De Clerck sp. nov.

### Diagnosis

Plantae usque ad 23 cm altae, crescentes ex haptero fibroso e partibus robustis teretibus constanti. Rhizoidea cylindrica, ad apices haptera adligantia aspergilliformia producentia. Axis inferior cylindricus rigidus usque ad 6 mm in diametro. Ramificatio usque ad quarties dichotoma, in partibus distalibus frequenter pinnata, regularis, opposita ad alternata. Rami alati, segmentis leviter angustatis vel dilatatis, costa distincta fere ad apicem (pinnis ultimis exceptis), 2–3 mm lati, pinnis angustioribus, nunquam latiores quam rami sui sustentantes. Pinnae angustae, breves (usque ad 3 mm longae) et in partibus distalibus arcte dispositae (distantes < 1.3 mm a se). Apices ramorum plerumque tricuspidati, cuspidem media cuspidem angustiore utroque latere aequanti vel eis brevior. Proliferationes superficiales plerumque numerosae. Structura vegetativa 4-strata. Cellulae corticis exterioris quadrangulares, cingentes zonam distinctam rhizinarum parietibus crassis a seriebus anticlinalibus cellularum corticis exterioris perductam. Cortex interior 2–3-stratus, cellulis periclinalibus, rotundis ad ellipticis. Medulla filamentosa, cellulis 6–10  $\mu$ m in diametro, cellulis majoribus (15–20  $\mu$ m) interspersis. Cystocarpia bilocularia, ad vel prope apices ramulorum lateralium portata, protuberantia distali instructa. Plantae masculae vel tetrasporophyta ignota.

Plants up to 23 cm tall, arising from a fibrous holdfast of robust, terete parts. Cylindrical rhizoids produce brush-like attachment haptera at their ends. Lower axis cylindrical, rigid and up to 6 mm in diameter. Up to four orders of distichous branching. Branches alate, tapered or broadened slightly in sections, with a distinct midrib ending just short of the apex, except in the ultimate pinnae. Branches 2–3 mm broad, pinnae narrower. Branches never broader than their bearing branch. Pinnate branching frequent in distal parts, regular, and opposite to alternate. Pinnae narrow, short (up to 3 mm long) and closely spaced (< 1.3 mm apart) in distal parts. Branch apices usually tridentate: middle tooth bordered on either side by narrower teeth and not extending beyond them. Numerous surface proliferations usually present. Plants have a four-layered vegetative structure. Outer cortical cells quadrangular, surrounding a distinct band of thick-walled rhizines traversed by anticlinal rows of outer cortical cells. Inner cortex two to three layers thick, cells periclinally arranged, round to elliptical. Medulla filamentous, cells 6–10  $\mu$ m in diameter, with larger cells (15–20  $\mu$ m) interspersed. Cystocarps bilocular, borne terminally or subterminally

on lateral branchlets and with a distal protuberance. Male or tetrasporophytic plants unknown.

ETYMOLOGY: This species is named in honour of Prof. Eric Coppejans of the Phycology Research Group of the University of Ghent, in recognition of the four decades of research he has conducted on the taxonomy of Indian Ocean seaweeds.

HOLOTYPE: GENT, KZN 1858 (Fig. 15), consisting of two thallus fragments, collected by R.J. Anderson and J.J. Bolton on 29 June 2000.

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). Protea Banks, The Caves, – 38 m; Tronchin; 10 ix 2003; ET 21 (BOL).

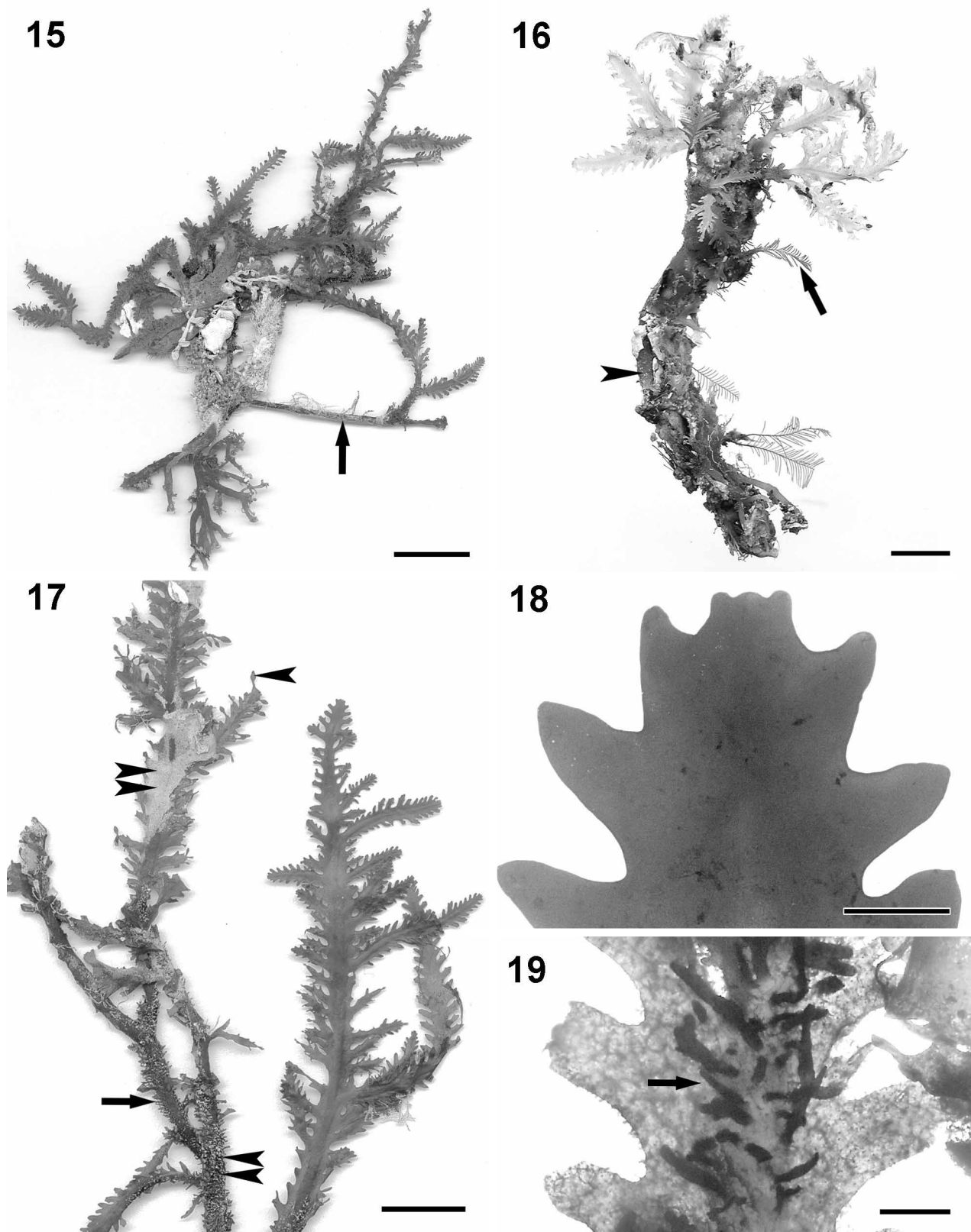
### Ecology

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 – 38 m. It forms expansive and almost monospecific stands around the perimeter of the two caves at Northern Pinnacle. 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. 15), which terminate in brush-like attachment haptera composed of aseptate, elongate cells. The lower axis is cylindrical, rigid, usually 2–3 mm wide, though axes as wide as 6 mm can be encountered (Fig. 16). Plants have up to four orders of pinnate branches. 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. 15). Branches of all orders, except ultimate pinnae, have a distinct midrib to just short of the apex and are pinnatifid. Branches are





**Figs 15–19.** *Ptilophora copejansii*. Fig. 15. One of two fragments of the holotype of *Ptilophora copejansii*. The axis and major branches are covered in a variety of epiphytes. Lower branches may become bare with age (arrow). Fig. 16. An aberrant morph with an axis (arrowhead) that is 6 mm in diameter. The axis is also covered in epiphytic sponges and hydroids (arrow). Fig. 17. Distal pinnate branches. An aberrant morphology 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 epiphytes. Sponge epiphytes also occur on parts of the frond with the normally encountered branch morphology (double arrowheads). Cystocarpic branchlets (single arrowhead) are borne on several pinnae. Fig. 18. Surface view of a branch apex. Fig. 19. 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 bars represent: Fig. 15, 2 cm; Fig. 16, 1 cm; Fig. 17, 1 cm; Fig. 18, 1 mm; Fig. 19, 1 cm.

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 spaced (< 1.3 mm apart) (Figs 15, 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 the formation of branch primordia. The apices of pinnae-bearing axes tend to be tridentate, the middle tooth being broadest, flanked on either side by narrower teeth (Fig. 18). Surface proliferations, which are simple and ligulate to 3 times pinnate, are regularly produced distal to the subapical parts (Fig. 19).

This species commonly develops aberrant morphologies. Some thalli may produce numerous fine, lanceolate (sometimes pinnate) proliferations in close proximity on the margins (Fig. 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 growth (Fig. 20), 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. 21).

#### *Vegetative structure*

Plants have a four-layered vegetative structure (Fig. 22). In transverse sections of second-order branches, the outer cortex consists of heavily pigmented, quadrangular cells. The cells are 8–10  $\mu\text{m}$  in length, 5–6  $\mu\text{m}$  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  $\mu\text{m}$  in diameter. The inner cortex consists of two to three layers of periclinally arranged, round or elliptical cells in transverse section. Cells are from 20 but mostly between 25–40  $\mu\text{m}$  in length, 14–30  $\mu\text{m}$  wide, with a length/width ratio of 1.2–1.9. Medullary filaments are 6–10  $\mu\text{m}$  in diameter with larger cells (15–20  $\mu\text{m}$ ) interspersed. Rhizines may occur towards the periphery of the medulla.

#### *Reproduction*

Fertile structures are borne terminally or subterminally on branches. Cystocarpic branchlets are

acuminate proximally and have a distal protuberance (Fig. 17). Cystocarps are bilocular and have one depressed ostiole per locule (Fig. 23). Male or tetrasporophytic plants have not been found.

#### *Epiphytes*

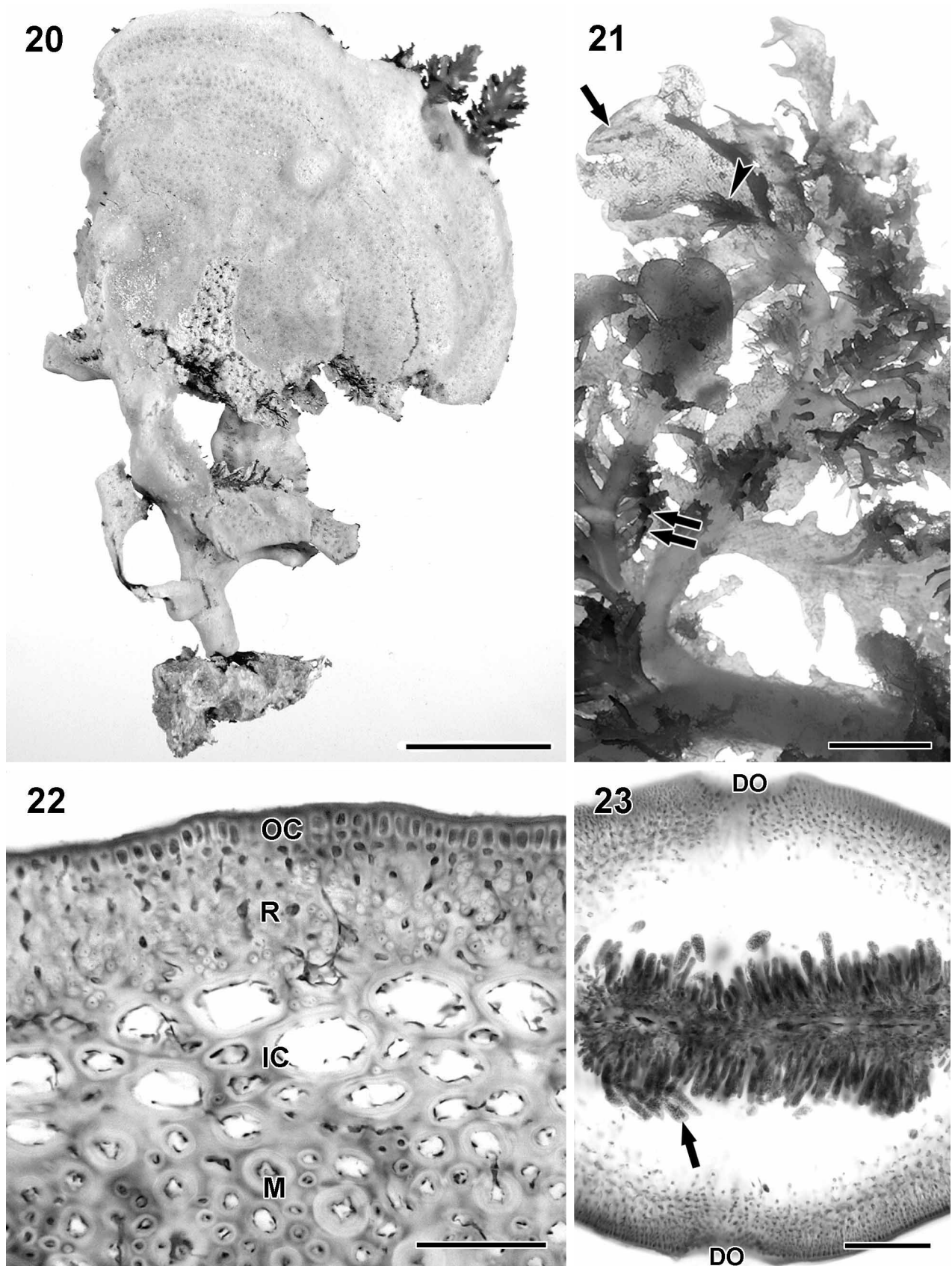
*Ptilophora coppejansii* is commonly associated with sponge and hydroid epiphytes. In some cases of sponge epiphytism, the association can be so extensive that the seaweed can easily be mistaken for a sponge.

#### *rbcL Analysis*

The analysed *rbcL* data set included 18 taxa and 1400 nucleotide sites. A total of 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. 24). Its topology was nearly identical to one of two most parsimonious trees of 424 steps generated by the parsimony analysis. These differed only in the placement of *Ptilophora ptero-cladioides* Andriamampandry, 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 *Ptilophora mediterranea* (H. Huvé) R. Norris, *P. pectinata* from Australia and *P. pterocladoides*. 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.

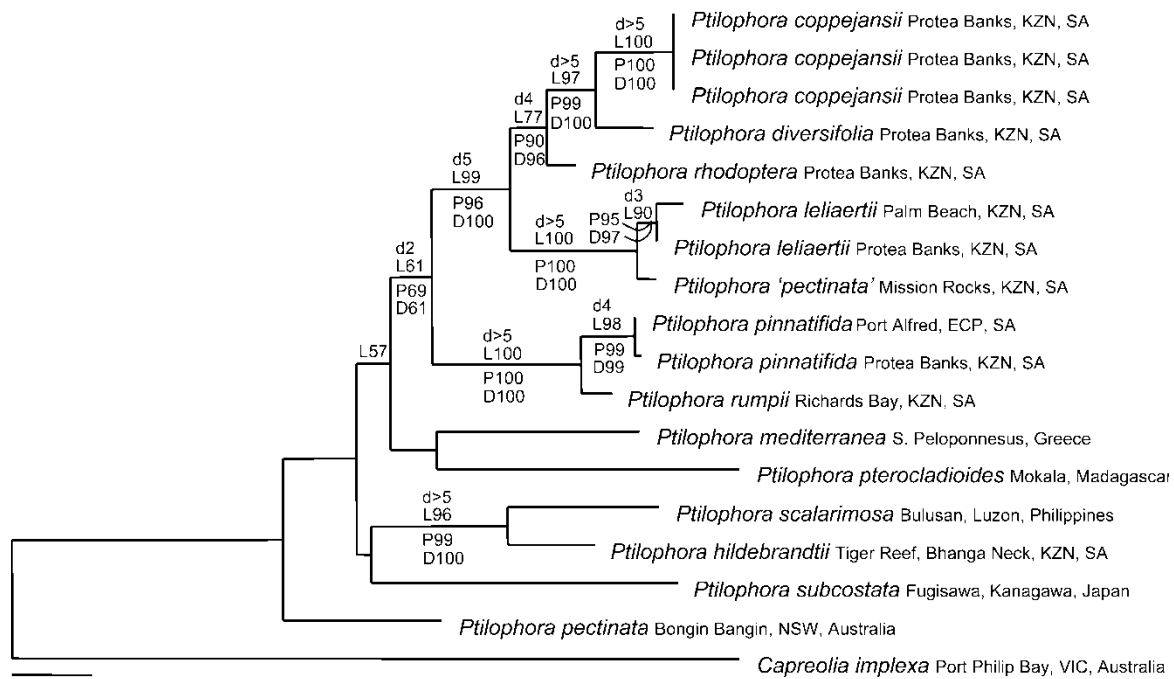
The analysed LSU data set included 13 taxa and 1143 sites. A total of 56 Sites (4.7%) were variable of which 15 (1.3%) were parsimony-informative. The data set had an unequal frequency of bases (A = 23.4%; C = 22.1%; G = 31.2%; T = 23.3%), a transition:transversion ratio of 1.85, and a 55.8% bias towards purine-purine transitions. A maximum likelihood analysis of these data produced four trees. A Shimodira-Hasegawa test did not find significant differences between the inverse log likelihood values of the four trees, therefore, the tree that had an identical topology to one of the 50 minimal trees of 66 steps found in the parsimony analysis is considered here (Fig. 25). This tree had a likelihood value of –2010.42. It differed slightly from the topology of the neighbour joining tree where *P. mediterranea*, *P. pectinata* from Australia, and *P. pterocladoides* were resolved as basal



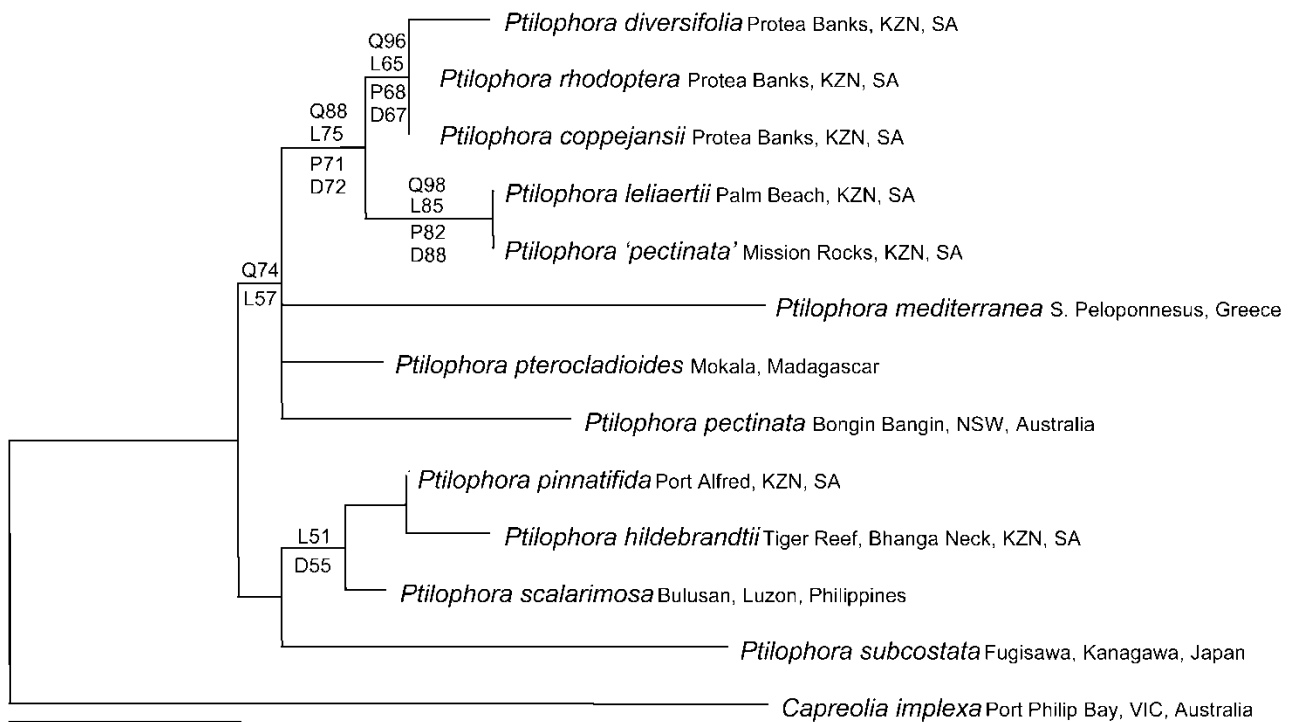


**Figs 20–23.** *Ptilophora copejansii*. Fig. 20. A plant that is completely enveloped by an epiphytic sponge except for the holdfast and a few distal branch tips. Fig. 21. A lateral branch of a totally sponge-encrusted plant with the sponge encrustation removed. The branch morphology is aberrant, characterized by abnormal branch apices (single arrow), extensive surface proliferations that are sometimes in tufts (arrowhead), and fine, closely spaced lateral branching (double arrows). Fig. 22. Transverse section of a second order branch. Outer cortex (OC); inner cortex IC; rhizine band (R); medulla (M). Fig. 23. Transverse section of a biloculate cystocarp with elongate carpospores (arrow) produced from the placental mass. Each locule has one depressed ostiole (DO). Scale bars represent: Fig. 20, 2 cm; Fig. 21, 3 mm; Fig. 22, 50  $\mu$ m; Fig. 23, 100  $\mu$ m.





**Fig. 24.** Maximum likelihood tree constructed from 18 aligned *rbcL* 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 expected nucleotide substitutions. Estimates of branch support provided include decay indices (d) as well as bootstrap support (%), when  $\geq 50$  (L = maximum likelihood; P = parsimony; D = distanced). Eastern Cape Province (ECP); KwaZulu-Natal Province (KZN); New South Wales (NSW); South Africa (SA); Victoria (VIC). The sequences for *P. mediterranea* and *P. pterocladoides* were obtained from isotype and holotype specimens, respectively. Scale bar represents: 0.01 substitutions per site.

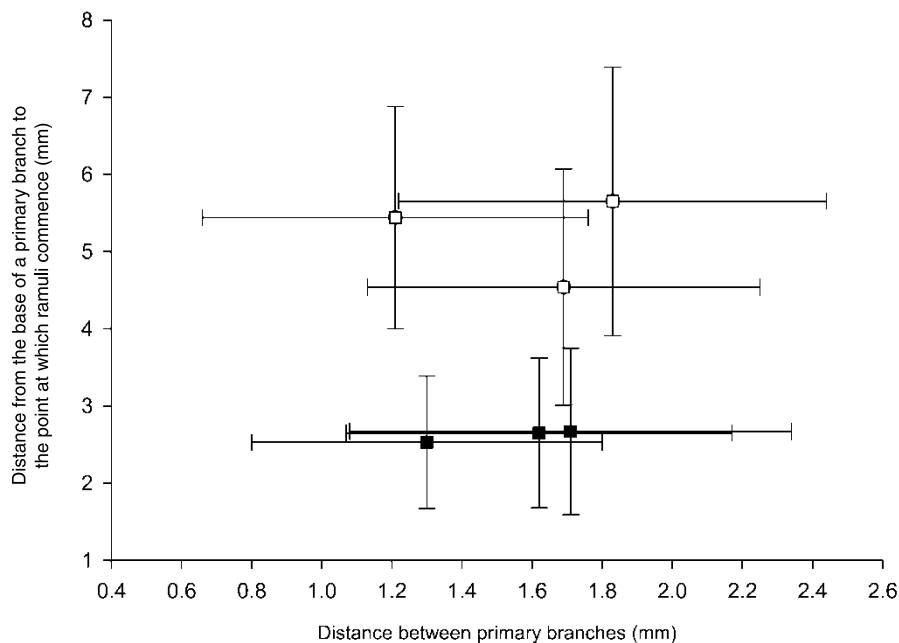


**Fig. 25.** Maximum likelihood tree constructed from 14 aligned LSU sequences from *Ptilophora* species using the Hasegawa-Kishino-Yano (1985) model and a ti:tv = 1.853. Branch lengths are proportional to the number of the expected nucleotide substitutions. Bootstrap support (%) and quartet puzzling reliability values are given for branches when  $\geq 50$  (Q = quartet puzzling; other abbreviations as in Fig. 24). The sequences for *P. mediterranea* and *P. pterocladoides* were obtained from isotype and holotype specimens respectively. Scale bar represents: 0.005 substitutions per site.

**Table 2.** Morphological characters of six species of *Ptilophora*

	<i>Ptilophora diversifolia</i>	<i>Ptilophora rhodoptera</i>	<i>Ptilophora copejansii</i>	<i>Ptilophora leliaertii</i>	<i>Ptilophora helenae</i> <sup>a</sup>	<i>Ptilophora pectinata</i>
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	✓	✓	✓	×	×	×
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	×	×	✓	×	✓	✓
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	with blunt or mucronate serrations	entire	entire	entire	entire
Shape of ultimate pinnae	not pinnate	not pinnate	linear with rounded/acute tips broadly and bluntly tridentate	linear with obtuse/acute tips acute to tridentate	rostrate to acuminate tridentate	rostrate to acuminate tridentate
Shape of indeterminate branch apex	rounded	rounded, retuse to emarginate				
Dentate proximal axis margins	×	×	×	×	✓	✓
Branching pattern	irregularly & sparsely branched	irregularly & sparsely branched	pinnate, sparse indeterminate branches	compound pinnate	compound pinnate, axes procurent	compound pinnate, axes procurent

<sup>a</sup>Referred to in the text as *Ptilophora 'pectinata'* from South Africa.



**Fig. 26.** The means and standard deviations of two dimensions measured from specimens of *Ptilophora pectinata* from Australia (□) and *Ptilophora* 'pectinata' from South Africa (■).

lineages. As with the *rbcL* analyses, there was weak to no support for the arrangement of basal lineages.

All *rbcL* trees generated by distance, maximum parsimony and maximum likelihood methods included a clade containing *P. copejansii*, *P. diversifolia*, *P. leliaertii*, *P. 'pectinata'* from South Africa and *Ptilophora rhodoptera* R. Norris. The sister relationships within this clade were always identical. Within this clade, three separate plants of *P. copejansii* collected from different locations on Protea Banks (two from Southern Pinnacle and one from Salmon Banks) had identical *rbcL* 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 copejansii* 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 *rbcL* 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 *rbcL* 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 *rbcL* and LSU data sets, *P. 'pectinata'* from South Africa was 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 2) between *P. 'pectinata'* from South Africa and *P. pectinata* from Australia, a morphometric investigation of two characters revealed that these specimens differed consistently in the distance from the junction with the axis to the point on primary branches at which ramuli commence. This distance is larger in plants from Australia (Fig. 26).

## 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 time evolutionarily, and neither *rbcL* or LSU gene sequences are able to resolve them clearly.

All *rbcL* analyses provide strong support for the recognition of *P. copejansii* and *P. leliaertii* as distinct species. LSU analyses did not support this, but LSU sequences are more conserved than *rbcL* sequences among Gelidiales taxa and may not vary between closely related species (Freshwater & Bailey, 1998; Freshwater *et al.*, 1999). The difference between *rbcL* 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 & Rueness, 1994). However, species have



been recognized previously despite their *rbcL* sequences being  $\leq 0.3\%$  divergent from their sister taxa (Shimada *et al.*, 1999, 2000). Sequence divergence can be useful as a guide to 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, and at rates that differ among 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 *rbcL* 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 procurent 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 2), with linear pinnae, and normally obtuse apices. Furthermore, the distributions of these two species are likely to be quite disjunct. *Ptilophora 'pectinata'* has been collected only from Mission Rocks and drift at Richards Bay (just south of Mission Rocks). Drift at Richards 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.

*Ptilophora 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. copejansii*, though, in 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 that are 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 copejansii* is distinguished by long major branches and sparse, irregularly arranged indeterminate laterals in distal parts, with a clearly visible midrib flanked 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 2).

*Ptilophora 'pectinata'* from South Africa and *P. pectinata* from Australia are distinct in molecular terms, but practically identical in morphology (Table 2). Plants from these two highly disjunct geographical regions share the same diagnostic characters of rostrate to acuminate determinate laterals and procurent axes and major branches. Vegetative structure, branching pattern, apical morphology, and thallus proportions such as branch width (Table 2) and between branch distances (Fig. 26), 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. 26). 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.

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