

***Pinnatiphycus menouana* gen. et sp. nov. (Rhodophyta: Dicranemataceae) from New Caledonia and Fiji (South Pacific): vegetative and reproductive morphology and molecular phylogeny**

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A new tropical genus and species belonging to the family Dicranemataceae, *Pinnatiphycus menouana*, is described from lagoon and outer reef-slope habitats in New Caledonia and Fiji. The new genus differs from other members of the family by the unique combination of the following characters: (1) tetrasporangia borne in terminal nemathecia on lateral cylindrical branchlets; and (2) the disposition of cystocarps along lateral branchlets rather than on the main axis itself. The new species differs from *Peltasta australis* J. Agardh by the presence of cylindrical lateral branchlets along the flattened main axes and the occurrence of reproductive structures in terminal, subterminal or basal positions on the lateral branchlets. It differs from subtropical *Reptataxis rhizophora* (Lucas) Kraft from Lord Howe Island by the presence of both yellowish refractive medullary cell clusters and cylindrical lateral branches bearing subapical tetrasporangial sori and cystocarps, as well as a central fusion cell and the production of carposporangia in chains of two to three rather than four to six. *rbcL* molecular analysis of Fijian samples unequivocally places the genus in the family Dicranemataceae with 100% bootstrap support, strongly relating it to two species of *Tylopus*. The family itself, however, received only weak bootstrap support (66%) for distinguishing it from the clade containing the virtually Australian-endemic families Mychodeaceae and Acrotylaceae. *Pinnatiphycus* favors deepwater habitats (65–70 m) with low light intensities or shallower (< 30 m) but turbid high-current areas, which may have contributed to it being overlooked in the past.

KEY WORDS: Dicranemataceae, Fiji, New Caledonia, Molecular phylogeny, Morphology, New genus, New species, *Pinnatiphycus*, *Pinnatiphycus menouana* sp. nov., *rbcL*, Reproduction, Rhodophyta, Taxonomy

INTRODUCTION

The Dicranemataceae (Kylin 1932, p. 65) is a largely southern Australian family for which the taxonomy has been revised and reviewed in detail by Kraft (1977) and Kraft & Womersley (1994). The family currently consists of four genera (*Dicranema* Sonder 1845, p. 56, *Peltasta* J. Agardh 1892, p. 102, *Reptataxis* Kraft 1977, p. 239 and *Tylopus* J. Agardh 1876, p. 428) and six species, four of which are endemic to southern Australia, one from Lord Howe Island and one from Japan and Taiwan. One species of *Dicranema*, *D. rosaliae* Setchell et Gardner, has been studied by Kraft (1977, p. 235), and found to have a *Gelidiopsis* or *Wurdemannia* habit and vegetative structure, rather than those characteristic of the genus *Dicranema*. Norris (1987) proposed that the Dicranemataceae should be merged with the Sarcodiaceae, but Liao *et al.* (1993) and Kraft & Womersley (1994, p. 321) argued convincingly for their maintenance as separate families on the basis of reproductive morphology and biochemistry, a conclusion strongly supported by the molecular evidence of Saunders *et al.* (2004). The Dicranemataceae is characterised by multiaxial thallus construction, monoecious gametophytes with two- or three-celled outwardly directed carposogonial

branches, diploidized auxiliary cells that form a small irregular fusion cell that cuts off multiple gonimoblast initials, spermatangia in sunken clustered pits in the outer cortex, and tetrasporophytes with zonate tetrasporangia. Cystocarps are protuberant, usually ostiolate and have a thick pericarp. In this paper we report a new genus and species of marine alga from New Caledonia and Fiji (South Pacific) with morphological and reproductive characters as well as *rbcL* sequences that place it in the Dicranemataceae, although its combination of characters differs from those of any other genus in the family.

MATERIAL AND METHODS

Morphological analyses

Specimens were collected by use of self-contained underwater breathing apparatus or dredging; some were stored in 5% buffered formalin in seawater, some in silica gel, and others were dried as herbarium specimens. Dried material was rehydrated in weak detergent solution before sectioning using a freezing microtome. Sections were stained using 1% acidified aniline blue and made permanent if necessary by mounting in 60% clear corn syrup. Drawings were made using a microscope with a camera-lucida attachment. Macrophotographs were tak-

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Table 1. List of species used in *rbcL* analyses with GenBank accession numbers.

Species	Location and collecting data	Accession no.	Source
<i>Acrotylus australis</i> J. Agardh	Port Macdonnell, South Australia (<i>M.H. Hommersand</i> , 28.vii.1995)	DQ343617	this study
<i>Antrocentrum nigrescens</i> (Harvey) Kraft & Min-Thein	Destrees Bay Road, Kangaroo I., South Australia (<i>M.H. & F. Hommersand</i> , 8.xi.1995)	DQ343618	this study
<i>Caulacanthus ustulatus</i> (Turner) Kützing	Swakopmund, Nairobi	AF099687	Fredericq <i>et al.</i> 1999
<i>Dicranema revolutum</i> (C. Agardh) J. Agardh	Port Macdonnell, Australia	AY294379	Gavio <i>et al.</i> 2005
<i>Hennedyia crispa</i> Harvey	Cervantes, West Australia (<i>M.H. & F. Hommersand</i> 20.ix.1995)	DQ343619	this study
<i>Mychodea hamata</i> Harvey	Port Macdonnell, South Australia	AF212191	this study
<i>Mychodea marginifera</i> (Areschoug) Kraft	Port Macdonnell, South Australia (<i>M.H. Hommersand</i> , 14.vii.1995)	DQ343620	this study
<i>Mychodea pusilla</i> (Harvey) J. Agardh	Queenscliff, South Australia (<i>M.H. & F. Hommersand</i> , 7.xi.1995)	DQ343621	this study
<i>Pinnatiphycus menouana</i> N'Yeurt, Payri et Gabrielson	Suva Reef, Fiji (<i>A.D.R. N'Yeurt</i> , 13.xi. 1999)	DQ343622	this study
<i>Sarcodia marginata</i> J. Agardh	Australia	AF212193	this study
<i>Sarcodia montagneana</i> (Hooker & Harvey) J. Agardh	Taipa, New Zealand (<i>W. Nelson</i> , 12.ii.1993)	AY294374	Gavio <i>et al.</i> 2005
<i>Trematocarpus flabellatus</i> (J. Agardh) De Toni	Kommetjie, Cape Peninsula, South Africa	AF385662	Hommersand & Fredericq 2003
<i>Tylotus lichenoides</i> Okamura	Kenting National Park, South Taiwan (<i>S.M. Lin</i> , 22.xi.1996)	DQ343623	this study
<i>Tylotus obtusatus</i> (Sonder) J. Agardh	Perth, Western Australia (<i>G.T. Kraft & J. Huisman</i> , 25.v.1996)	DQ343624	this study

en with a Nikon E-995 digital camera (Nikon Corp., Tokyo, Japan); photomicrographs were obtained using an Olympus BH2 compound microscope fitted with an Olympus C-4040 or C-5050 digital camera (Olympus Optical Co., Tokyo, Japan), and the resulting files processed into figures by computer software. Voucher specimens have been deposited in the herbaria of the Université de la Polynésie Française in Tahiti (UPF) and IRD (Nouméa, New Caledonia). Accession numbers preceded by the letter S refer to microscope slide collections.

Molecular analyses

Table 1 shows the taxa included in the molecular analyses, along with their localities, collection data and GenBank accession numbers. Silica gel-dried material was extracted following the protocol in Hughey *et al.* (2001). The coding region of *rbcL* was amplified using the primer combinations F-57 and R-753 and F-753 and R-*rbcS* (Freshwater & Rueness 1994), the amplification and sequencing protocols being those of Hughey *et al.* (2001). Sequences were compiled and manually aligned using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and Sequence Alignment Editor available at <http://evolve.zoo.ox.ac.uk/Se-Al/Se-Al.html>. Maximum parsimony (MP) and neighbor joining (NJ) analyses were performed using PAUP (v. 4.0b10, Swofford 2002). Of the 1467 base pairs (bp) of the *rbcL* gene, 1344 were used in the analysis. In the MP analysis all characters and character changes were weighted equally. Heuristic searches of 500 replicates of random sequence additions were done with Multrees and Tree-Bisection-Reconnection in effect. NJ and MP bootstrap analyses consisted of 2000 replicates of full heuristic searches.

OBSERVATIONS

Pinnatiphycus N'Yeurt, Payri et Gabrielson, gen. nov.

Thallus basin versus subcylindricus stolonifer, supra complanatus, ramificatione subdichotoma (infra) vel dichotoma (supra), ad substratum per discum basalem et haptera plura cylindrica e pagina inferiore sub angulo 90° abeuntia affixus. In axibus maturis medulla ex filis elongatis materia contenta lutea refractiva plerumque, in utroque latere cellulis medullosis grandibus rotundatis circumcincta; extus cellulae hae gradatim deminuentes, medullo gradatim in corticem ex cellulis parvis radialiter elongatis constatum transienti. Plantae monoicae. Rami carposporangiales tricellulares respectu paginam thalli directi, in locis tumidis corticis secus ramos laterales cylindricos ubique dispositi. Species verisimiliter sine procarp. Carposporophyton sphaericum, conjuncticellula parva centrali fila gonimoblasti radialia ferenti, his in catenas carposporangiorum 2–3 terminantibus respectu paginam thalli directas. Cystocarpia tumida subterminalia ostiolis latis, 1–3 in ramis lateralibus in medio vel ad basin posita. Spermatia aggregata bina vel terna, gregibus his inter se sejunctis, in areis depressis corticis ramorum lateralium cylindricorum cystocarpiferorum utrinque dispositis. Tetrasporangia zonata, paraphyse clavata sterili semel divisa, inter cellulas corticales in partibus subterminalibus parum tumidis ramorum lateralium cylindricorum dispersa.

Thallus basally subcylindrical and stoloniferous, upper parts flattened, complanate, subdichotomously (lower) to dichotomously (upper) branched, attached to the substratum via a basal disc and several cylindrical haptera issued at right angles from the inferior surface. Mature axes with a filamentous medulla of elongate clusters of cells mostly with yellow refractive contents surrounded on either side by large rounded medullary cells becoming progressively smaller and grading into a layer of small radially elongate cortical cells. Plants monoecious; carpoogonial branch three-celled, oriented straight to the thallus surface, occurring in locally swelled areas all along the cortex of cylindrical lateral branchlets. Presumably non-procarpic. Carposporophyte spherical, with a small central fusion cell, bearing radial gonimoblast filaments terminating in chains of

two to three carposporangia oriented toward the surface. Cystocarps globular and with wide ostioles, occurring singly or in clusters of two to three in subterminal, median or basal positions on cylindrical lateral branchlets. Spermatangia in isolated clusters of two to three, occurring in sunken areas of the cortex at the basal part of the cystocarpic lateral branchlets. Tetrasporangia zonate, each associated with once-divided club-shaped sterile paraphyses and scattered among normal cortical cells in slightly swollen terminal areas of lateral branchlets.

ETYMOLOGY: *Pinnatiphycus* is named for the terete, pinnately arranged unbranched laterals that conspicuously characterize the thalli.

TYPE AND ONLY SPECIES: *Pinnatiphycus menouana* N'Yeurt, Payri & Gabrielson.

***Pinnatiphycus menouana* N'Yeurt, Payri & Gabrielson,
sp. nov.**

Figs 1–19

Plantae 3–20 cm longae, axem principalem (1-) 1.3–1.5 (-3.5) mm latae, crassitie 300–320 μm , apicibus obtuse rotundatis. Rami laterales angusti cylindrici et irregulatim regulatimve oppositi pinnati, (1-) 2–3 (-6) mm longi, (220-) 480–490 (-560) μm lati secus axem principalem per spatia 350–500 (-1000) μm dispositi.

Plants 3–20 cm long, main axes (1-) 1.3–1.5 (-3.5) mm wide and 300–320 μm thick, apices bluntly rounded. Narrow, irregularly to regularly opposite and pinnate cylindrical lateral branchlets (1-) 2–3 (-6) mm long and (220-) 480–490 (-560) μm wide proliferate at 350–500 (-1000) μm intervals along the main axes.

ETYMOLOGY: The specific epithet honors Jean-Louis Menou, who first collected the new species and who repeatedly dived up to 70-m depths to collect material for this study.

HOLOTYPE: IRD 0028/PC 0062760, monoecious gametophyte (Fig. 1), *leg. J.-L. Menou*, 10 Feb. 1983, 30 m depth, deposited in the Muséum National d'Histoire Naturelle, Paris.

TYPE LOCALITY: West inlet of Woodin Channel, Sirekoui Pt., New Caledonia (22°23.900'S, 166°46.800'E).

PARATYPES: **NEW CALEDONIA:** IRD 0033 (female, male and tetrasporophyte), IRD 0032/PC 0067761 (tetrasporophyte, male and female), Récif Mbere (ST 750), *leg. J.-L. Menou*, 12 Feb. 2004, 65 m depth; IRD 0036, IRD 0037, (male / female), IRD 0038 (tetrasporophyte), IRD 0039-UPF 3010 (female, male and tetrasporophyte), Récif Tomboo, outer slope (ST 720), *leg. J.-L. Menou*, 24 Mar. 2004, 65–68 m depth; IRD 0029, IRD 0030, IRD 0031 (female, male and tetrasporophyte), Récif Tomboo outer slope (ST 720), *leg. J.-L. Menou*, 2 Jul. 2004, 68 m depth; IRD 0035, Pass Tomboo (ST 748), *leg. J.-L. Menou*, 6 Feb. 2004, 50–65 m depth; IRD 0034, Chenal de la Havanna (ST 862), *leg. C. Payri*, 25 Nov. 2004, 41 m depth; IRD 0040 (female), Banc Ionontéa, Lagon Sud-Ouest (DW247), *leg. B. Richer de Forges & C. Garrigue*, 23 Oct. 1984, 29 m depth; IRD 0041 (tetrasporophyte), Canal Woodin (ST 332), *leg. G. Bargibant & J.-L. Menou*, 20 Mar. 1985, 30 m depth; IRD 0042 (tetrasporophyte), IRD 0043 (female), IRD 0044, Les 4 Bancs du Nord, Lagon Sud Ouest (DE69), *leg. B. Richer de Forges & C. Garrigue*, 2 May 1987, 35 m depth; IRD 0050, Poindimié, Plateau de Tyé (ST 764), *leg. C. Payri*, 5 May 2004, 30 m depth; IRD 0045, Baie de chateaubriand, Lifou, Loyalty (ST 629), *leg. C. Payri*, 24 Mar. 2005, 47 m depth; IRD 0046, Cap Bernardin, Lifou, Loyalty (ST631), *leg. C. Payri*, 5 Mar. 2005, 25 m depth; IRD 0047, Mare, Loyalty (ST 623), *leg. C. Payri*, 22 Mar. 2005, 36 m depth; IRD 0048, Ilôt Agnéhu, Ouvéa, Loyalty (ST 643), *leg. C. Payri*, 31 Mar. 2005, 21 m depth; IRD 0049, Beautemps-Beauprè, south point, Loyalty (ST 645), *leg. C. Payri*, 1 Apr. 2005, 25 m depth. **FJI:** UPF 2730 (female, carpogonial), Belcher Rocks, Suva Reef (18°11'040"S, 178°32'030"E), *leg. D. W. Keats*, 27 Nov. 1994, 30 m depth; UPF 2731 (male, female), UPF 2732 (tetrasporic), *leg. A. D. R. N'Yeurt*, 13 Nov. 1999, 25 m depth.

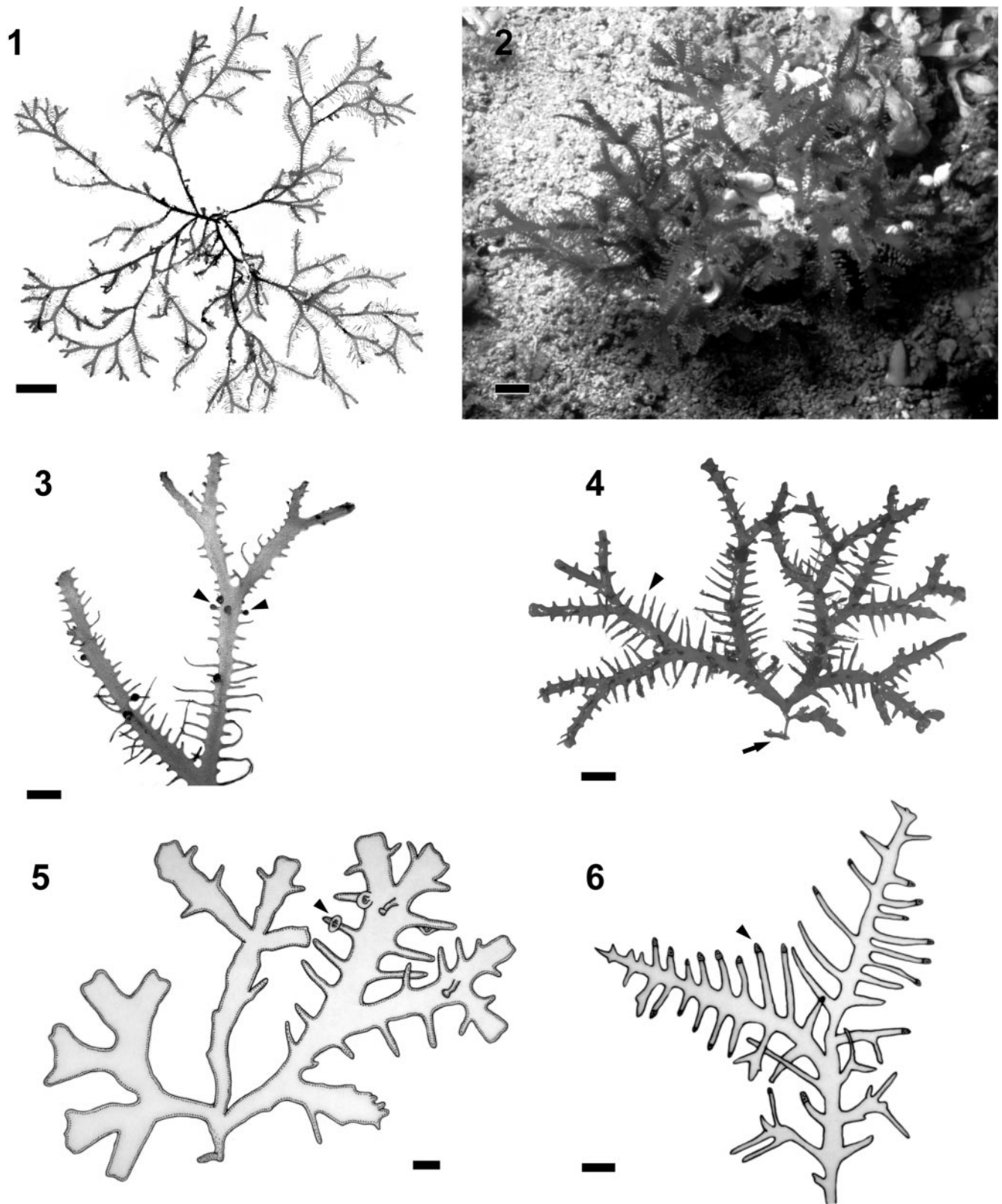
DISTRIBUTION: Known thus far only from south-western, eastern reefs

and lagoons of 'Grande-Terre' and Loyalty Islands, New Caledonia and the Suva Reef, Fiji.

HABITAT AND SEASONALITY: Collections were made from the same localities in Fiji in November 1994 and November 1999, and from New Caledonia in February 1983, October 1984, March 1985, July 2003, and February and March 2004. More recently, collections were made from the Loyalty Islands (N. E. of New Caledonia) in April 2005. All material was fertile thalli except that collected in July 2003. Thalli were relatively rare and found growing on coral debris and pebbles at depths of 15 to 70 m in the lagoon or on the outer reef slope. The New Caledonian material was growing under low light intensities between 65 and 68 m deep on the outer slopes of barrier reefs, or in particularly turbid areas with often strong currents in the south-west and east lagoons at depths of between 25 and 30 m. The Loyalty Island material was collected from shallower habitats (25-m depth). The Fijian site (Belcher Rocks, Suva, 30-m depth) was characterized by strong currents and high turbidity caused by mixing of salt and fresh water from the nearby Rewa River delta. The rocky outcrop on which plants were found lies just outside of the barrier reef, is subject to heavy wave action, and is only accessible by boat for a few days each year.

HABIT AND VEGETATIVE MORPHOLOGY: Plants are reddish-brown, generally complanate, and up to 10 times subdichotomously (below) to dichotomously (above) branched, with 3–20 cm long, flattened, main axes (1) 1.3–1.5 (3.5) mm wide and 300–320 μm thick (Figs 1–6), beset with abundant, pinnately arranged, usually simple, cylindrical laterals. Plants arise from a short, subcylindrical, simple stoloniferous axis 3–10 mm long attached to the substratum via a basal disc 1–2 mm in diameter, giving rise to 2–3 erect complanate axes. The complanate axes are closely attached to the substratum, usually rocky debris or pebbles (Fig. 2), via several sparsely distributed, cylindrical haptera about 480 μm in diameter that arise perpendicularly from the inferior surface, usually near the bluntly rounded and somewhat wider (to 3.5 mm) apices. Narrow, irregularly to regularly pinnately arranged, sometimes bifid cylindrical lateral branchlets (1-) 2–3 (-6) mm long and (220-) 480–490 (-560) μm wide occur at 350–500 (-1000) μm intervals along both sides, and sometimes on median portions of the main axes, but never attach to the substratum by terminal or lateral haptera. The internal structure (Figs 7–10) consists of a narrow medulla of elongate filaments 4–7 μm in diameter, the cells of which are 11–45 μm long with mostly yellow refractive contents (Figs 9, 10), the filaments in turn surrounded by spherical medullary cells 22–33 μm long and 13–22 μm wide with clear granulose contents. The outer medulla grades into a cortex of progressively smaller cells, the outermost cells being radially elongate, darkly pigmented, 3.3–4.5 μm wide and 6.7–11 μm long. Remains of cortical hairs with refractive, glandular basal cells (Fig. 8) are scattered in the surface. Plants, when fresh, have a reddish-brown colour and a cartilaginous, leathery texture with a smooth surface. Plants do not adhere well to paper and become papery in texture as they darken on drying.

REPRODUCTIVE MORPHOLOGY: In New Caledonian populations, tetrasporophytes are often more robust and have wider main axes than gametophytes. The opposite was observed in Fijian populations, which hints that environmental conditions



Figs 1–6. *Pinnatiphycus menouana* N'Yeurt, Payri & Gabrielson: habit of holotype and paratypes.

Fig. 1. Habit of holotype (IRD 0028/PC 0062760) from New Caledonia. Scale = 20 mm.

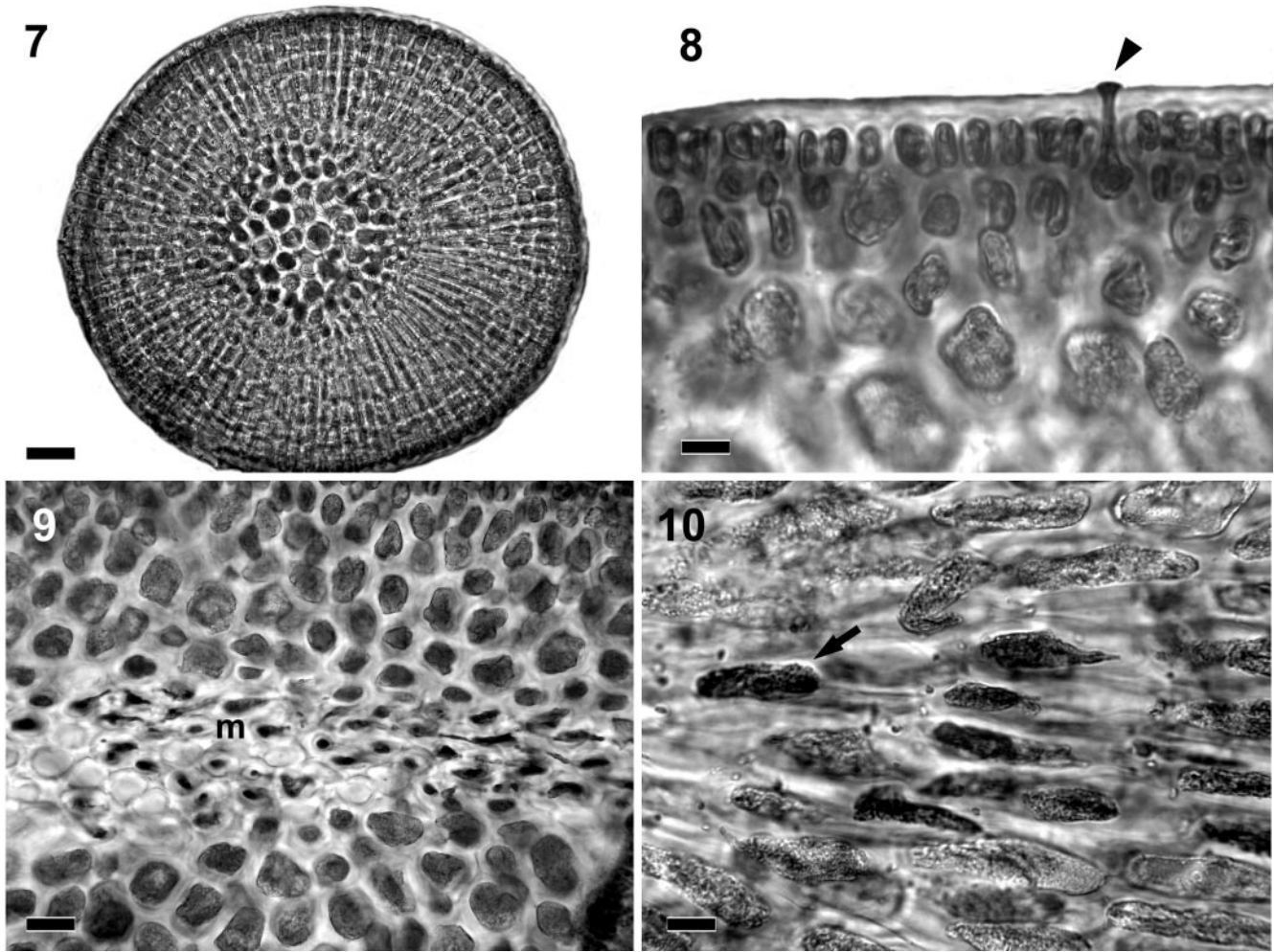
Fig. 2. *In situ* habit of holotype at –30 m. Scale = 6 mm.

Fig. 3. Detail of branch of holotype, showing cystocarps (arrowheads) on lateral branchlets. Scale = 4 mm.

Fig. 4. Habit of paratype (IRD 0038) from outer reef-slope habitat in New Caledonia, showing more compact form from holotype, and tetrasporic swelling on side branchlets (arrowhead). Note the cylindrical stoloniferous basal portion (arrow). Scale = 4 mm.

Fig. 5. Drawing of female paratype from lagoon habitat in Fiji (UPF 2731) showing cystocarp (arrowhead) and irregular occurrence of lateral branchlets. Scale = 1 mm.

Fig. 6. Drawing of paratype from lagoon habitat in Fiji (UPF 2732) showing narrower axes than a typical gametophyte, and subterminal tetrasporangial swellings on side branchlets (arrowhead). Scale = 2 mm.



Figs 7–10. *Pinnatiphycus menouana* N'Yeurt, Payri & Gabrielson: vegetative anatomy.

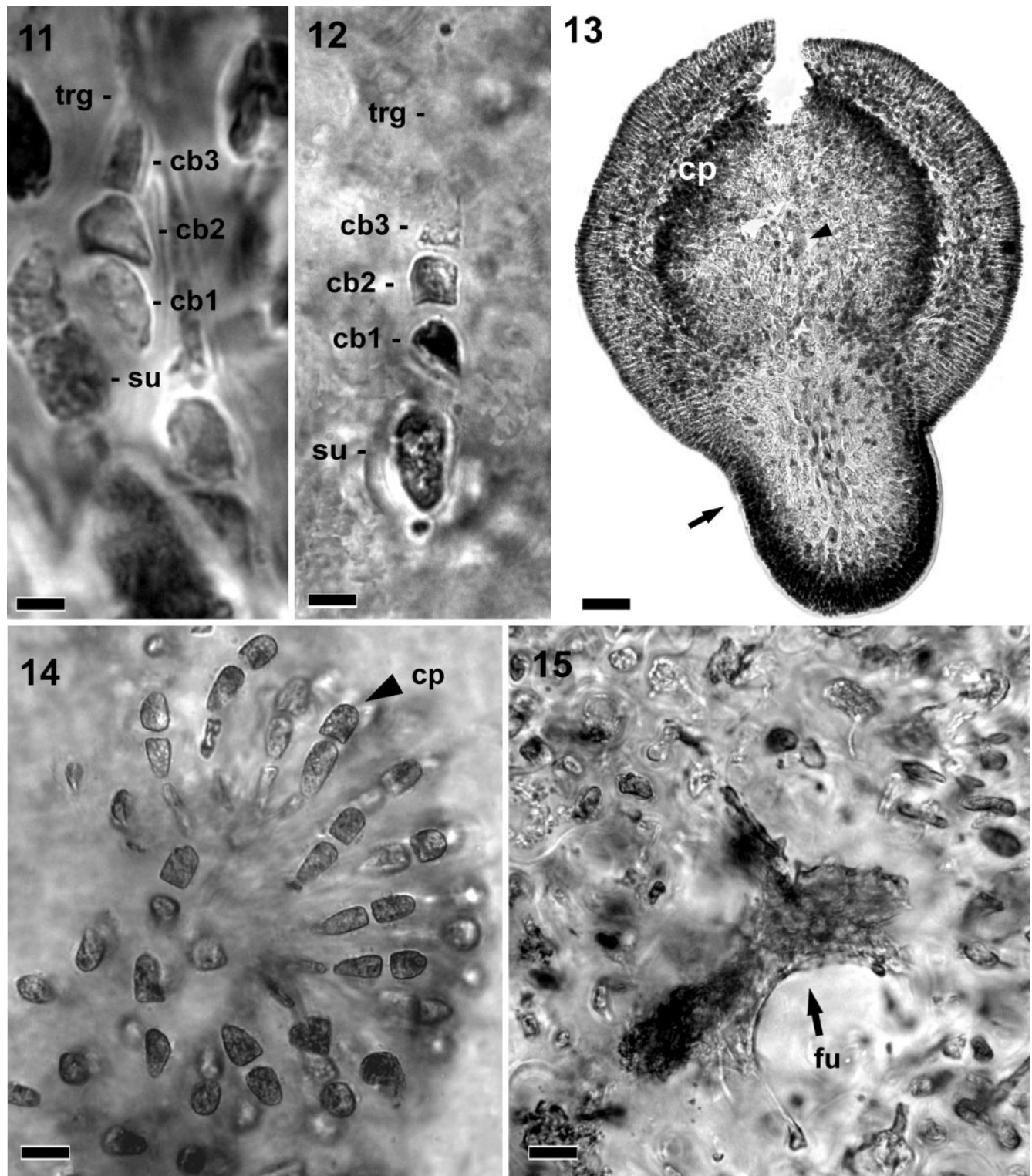
- Fig. 7.** Transverse section of cylindrical lateral branchlet, showing central region of refractive medullary cells (UPF 2731). Scale = 20 μm .
Fig. 8. Transverse section of cortex showing radially elongate cortical cells and remnants of a cortical hair (arrowhead) with a refractive, glandular basal cell (UPF 2730). Scale = 10 μm .
Fig. 9. Longitudinal section of thallus showing medullary region (m) of elongate filaments, some cells of which have refractive content, surrounded on either side by subisodiametric outer medullary cells (UPF 2730). Scale = 20 μm .
Fig. 10. Detail of medulla, showing stout, elongate filaments containing some cells with refractive content (arrow) (UPF 2731). Scale = 10 μm .

may affect morphogenesis in the species. Lateral branchlets are longer (up to 6 mm) on tetrasporophytes.

Gametophytes are monoecious. Carpogonial branches often are located within dome-like swellings that rise 35–45 μm above the surrounding frond surface, these being distributed along both sides of cylindrical lateral branchlets and sometimes forming just distal to areas in which spermatangia have developed. Each carpogonial branch is three-celled, bears a long trichogyne (Figs 11, 12) that is directed straight to the thallus surface, and is borne on an undifferentiated inner-cortical supporting cell. Cells of the carpogonial branch are 4–5 μm long, with the entire branch measuring 16–19 μm long (excluding the trichogyne). The species is probably non-procarpic, but this could not be confirmed. After presumed fertilization, the auxiliary cell apparently fuses with adjacent cortical cells and branches into the surrounding cortical filaments, forming a small outwardly directed fusion cell (Fig. 15). Multiple gonimoblast initials are cut off from the fusion cell, to

form a placenta of partly fused and pit-connected gonimoblast and vegetative cells. An ostiole progressively develops from the surface inward toward the developing carposporophyte. The carposporophyte is roughly spherical, with a central placenta of intermixed gonimoblast and vegetative filaments. Peripheral gonimoblasts become columnar and parallel, bearing chains of two to three carposporangia 5–6 μm in diameter and 5–8 μm long oriented toward the surface (Fig. 14). One to three mature cystocarps are located in subterminal, medial or basal positions on cylindrical lateral branchlets (Fig. 13). Initially subterminal cystocarps can become lateral, as the branch apices can continue growing. Cystocarps are protuberant, 360–600 μm in diameter by 360–500 μm in height, with a thick pericarp and an ostiole 54–60 μm wide. The number of cystocarps is variable, sometimes with as many as 18 per main axis or less than 10 for an entire plant.

Spermatangia occur in the basal portions of cystocarpic lateral branchlets proximal to carpogonial swellings. The male



Figs 11–15. *Pinnatiphycus menouana* N'Yeurt, Payri & Gabrielson: female reproductive anatomy.

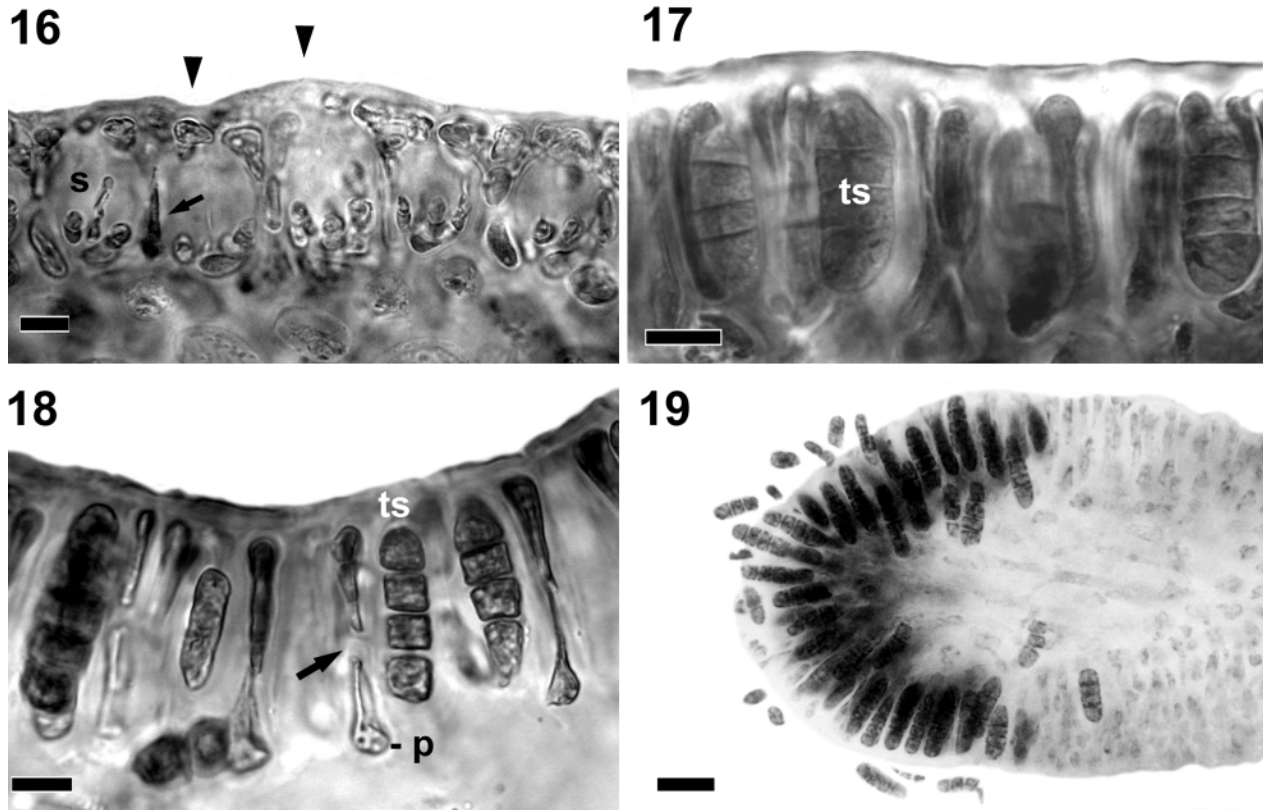
Fig. 11. Supporting cell (su) bearing a three-celled carpoogonial branch (cb 1–3) with trichogyne (trg) (UPF 2727). Scale = 5 μm .

Fig. 12. Orthostichous alignment of carpoogonial-branch cells directed toward the thallus surface (UPF 2730). Scale = 5 μm .

Fig. 13. Longitudinal section through an ostiolate mature carposporophyte, subterminal on a continuously growing secondary axis (arrow), with its radiating peripheral chains of carposporangia (cp) and central fusion cell (arrowhead) (UPF 2731). Scale = 20 μm .

Fig. 14. Detail of peripheral region of mature carposporophyte, showing carposporangia (cp) radiating in chains of two to three (IRD 0033). Scale = 10 μm .

Fig. 15. Detail of radiating central fusion cell (fu) of carposporophyte (UPF 2731). Scale = 10 μm .



Figs 16–19. *Pinnatiphycus menouana* N'Yeurt, Payri & Gabrielson: male and tetrasporangial anatomy.

Fig. 16. Longitudinal section through cluster of spermatangial pits (arrowheads) in outer cortex of the holotype, showing spermatangia (s) bordered by clavate paraphyses (arrow) (IRD 0028/PC 0062760). Scale = 10 μ m.

Fig. 17. Longitudinal section through tetrasporangial swelling on lateral branchlet of New Caledonian material showing relatively broad zonate tetrasporangia (ts) (IRD 0032). Scale = 8 μ m.

Fig. 18. Longitudinal section through tetrasporangial swelling of Fijian material showing relatively narrow tetrasporangia (ts) bordered by a single two-celled (arrow) clavate paraphysis (p) (UPF 2720). Scale = 10 μ m.

Fig. 19. Longitudinal section through distal portion of a tetrasporangial lateral on a plant from Fiji, the tetrasporangia densely scattered in outer cortex (UPF 2732). Scale = 20 μ m.

gametes are ovoid, 1.5–4 μ m in diameter, and develop singly or in pairs on two or three mother cells that surround inner-cortical bearing cells located at the base of ampullar pits 26–28 μ m high and 19–20 μ m wide, the pits bordered by clavate paraphyses (Fig. 16).

Tetrasporangia (Figs 17–19) are zonate, (4.5–) 7–8 (–11) μ m wide and (20–) 24–26 (–40) μ m long, and aggregated among outer cortical cells in slightly swollen, club-shaped subterminal areas of lateral branchlets. Sporangia are divided into four subquadrate tetraspores and are each associated with a sibling cell borne on the same parent cell and which is medially divided into a two-celled clavate paraphysis. The widths of tetrasporangia range from relatively broad (Fig. 17) to narrow (Figs 18, 19) in different thalli.

MOLECULAR ANALYSIS: The 1344 bp alignment of the *rbcL* gene sequence for all taxa contained no insertions or deletions; 487 characters were variable, of which 352 were parsimony informative. A single tree with the same topology was found in both the NJ (Fig. 20) and MP (not shown) analyses. In both analyses, *Pinnatiphycus* was unequivocally placed in the family Dicranemataceae and always grouped with *Tylopus* (100% bootstrap support), the two species of which have also been subject to *rbcL* analysis (Table 1). The family Dicranemata-

ceae, however, received only weak bootstrap support (66%) as separate from the Mychodeaceae and Acrotylaceae.

DISCUSSION

Morphological and reproductive evidence

The Dicranemataceae is a well-defined family within the Gigartinales with distinctive reproductive characters and some of the most complex carposporophytes in the red algae (Kraft 1977, 1981; Kraft & Womersley 1994). Kraft (1977) redefined the Dicranemataceae (as Dicranemaceae) to include those genera that shared with *Dicranema* similarities, particularly in regard to early gonimoblast development. In the four genera that Kraft (1977) placed in the family (*Dicranema*, *Peltasta*, *Reptataxis* and *Tylopus*), as is also true of *Pinnatiphycus*, the diploidized auxiliary cell apparently fuses directly with adjacent vegetative cells to form a small, central irregular fusion cell from which multiple, filamentous gonimoblasts are produced that are placentated across a broad base or central region, consist of an extensive central mass of sterile tissue, and produce single-terminal or chains of carposporangia periph-

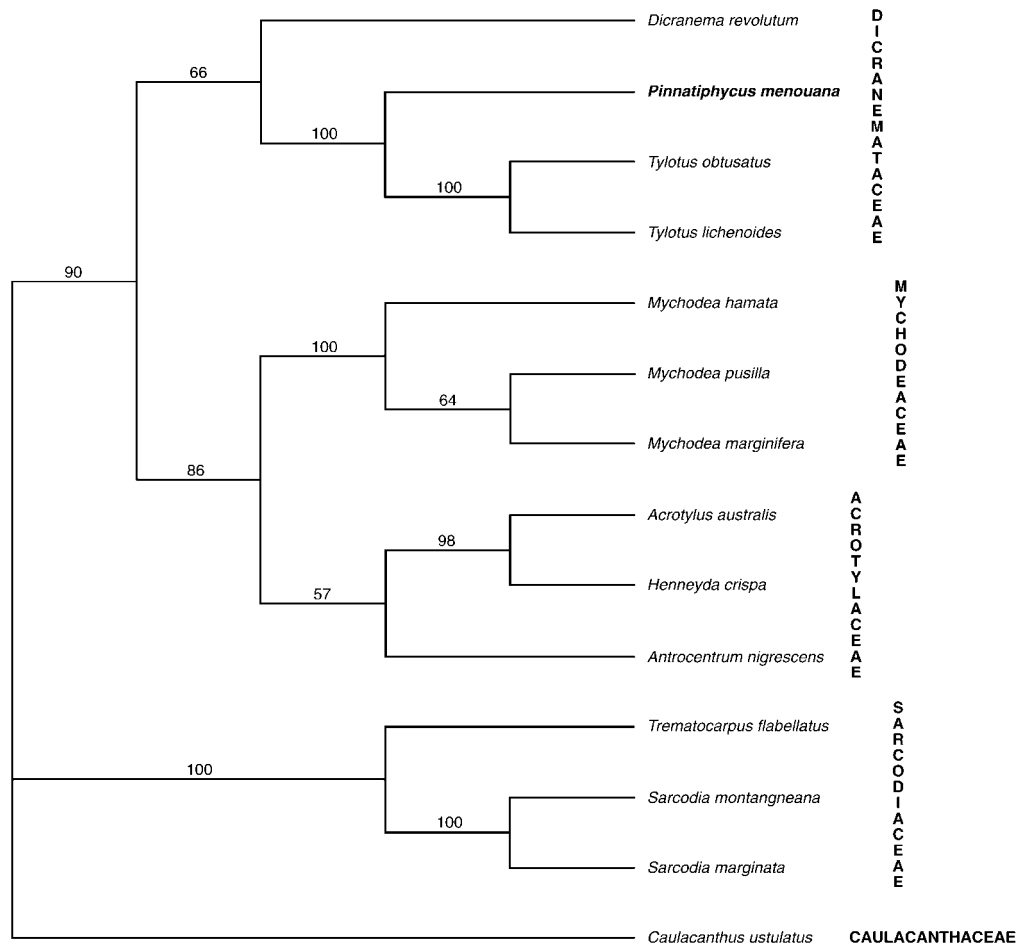


Fig. 20. NJ tree placing *Pinnatiphycus menouana* in the family Dicranemataceae. Numbers above branches indicate bootstrap support values.

erally. Additional features shared by all of these taxa are their multiaxial construction, monoecious gametophytes, outwardly directed carpogonial branches that lack sterile cells, thick ostiolate pericarps (with the exception of *Dicranema cincinnalis* Kraft), and zonately divided tetrasporangia. The vegetative and reproductive characters of *Pinnatiphycus* conform well to those of the family.

When comparing *Pinnatiphycus* with other genera of the Dicranemataceae (Table 2), it can be seen that it differs from all of them by the presence of abundant, typically unbranched, cylindrical lateral branchlets on which tetrasporangia are aggregated in terminal nemathecia, and cystocarps that occur in median, subterminal or basal swellings but never on the main axes themselves.

Among genera of the Dicranemataceae, the two species of *Dicranema* itself differ in a number of respects from *Pinnatiphycus*, including the restriction of both to woody stems of the seagrass *Amphibolis* spp. Other differences include their two-celled carpogonial branches, inwardly rather than outwardly oriented carposporophytes, either the lack of an ostiole or development of the ostiole on the branch opposite (rather than above) the fusion cell, and the single-terminal carposporangia (Kraft 1977).

Tylotus, with which the *rbcL* data strongly ally *Pinnatiphycus*, differs in its strap-like regularly dichotomous habit, ‘cellular’ (non-filamentous) medulla, separation of male and fe-

male gametangia on opposite sides of the complanate fronds, laterally rather than apically positioned carpogonial branches on supporting cells and broadly hemispherical carposporophytes covered by a convoluted surface layer of single-terminal carposporangia (Kraft 1977).

The only subtropical austral genus of Dicranemataceae is *Reptataxis*, at present still considered to be endemic to isolated Lord Howe Island in the Australasian South Pacific. As largely complanate, often imbricating thalli anchored by ventral terete haptera (as in *Pinnatiphycus*), as well as being the only other genus to display ‘glandular’ hairs and chained subisodiametric carposporangia (also as in *Pinnatiphycus*), it certainly appears to be the most closely related of all the genera. It differs, however, in lacking medullary cells with yellow refractive contents, and by possessing distinct subterminal tetrasporangial nemathecia, a basal fusion cell, and carposporophyte producing chains of four to six carposporangia (Kraft 1977).

It could be possible that features like glandular hairs are a synapomorphy, whereas refractive medullary cells are a symplesiomorphy, but only a full molecular analysis of the family would be apt to settle this issue. One interesting difference between *Pinnatiphycus* and *Reptataxis* is in their vertical distributions, that of *Reptataxis* ranging from the low-intertidal to just -8 m or so, whereas this is not found at the New Caledonian and Fijian depths of *Pinnatiphycus*.

Table 2. Comparison of selected characters between *Pinnatiphycus* – and other genera of Dicranemataceae¹.

	Medullary construction	Medullary refractive cells	Carpogonial branch	Carpophyte orientation	Fusion cell	Carposporangia	Cystocarp position	Tetrasporangia location	Tetrasporangial paraphyses	Spermatangia location	Spermatangial cluster paraphyses
<i>Pinnatiphycus</i>	filamentous	present	three-celled	outward	central	chains of two to three	only along lateral branchlets	in swollen apical nemathelial branchlets on lateral branchlets	divided	in sunken con- ceptacles	present
<i>Dicranema</i>	filamentous	absent	two-celled	inward	basal	single, terminal	at branch tips	in swollen apical nemathelial branchlets	absent	in clusters, on buried cortical cells	absent
<i>Peltasta</i>	filamentous	present	three-celled	outward	central	chains of two to three	at branch tips	scattered in cortex, subapical	undivided	in sunken con- ceptacles	present
<i>Reptataxis</i>	filamentous	absent	three-celled	outward	basal	chains of four to six	on the main axis or branch margins	in subapical nemathelial patches	absent	in clusters, on buried cortical cells	present
<i>Tylotus</i>	cellular	absent	three-celled	outward	basal	single, terminal	scattered on the main axis	in subapical nemathelial patches	absent	in sunken con- ceptacles	present

¹ Sources: Kraft 1977; this study.

Also sharing some features of the new genus is the monotypic Australian-endemic genus *Peltasta*, which, like *Pinnatiphycus*, is characterised by basal terete stolons that give rise to compressed erect axes with a narrow medulla of elongate cells with yellowish refractive contents, a three-celled, straight carpogonial branch, and subterminal to lateral, widely ostiolate cystocarps, and with a central fusion cell giving rise to gonimoblast initials bearing carposporangia in radiating chains of two to three oriented toward the surface (Kraft 1977). However, *Peltasta* differs from *Pinnatiphycus* in: (1) its cool-temperate distribution; (2) wider axes (2–3 mm broad) lacking cylindrical, lateral branchlets; (3) cystocarps at the branch tips, which then cease growth; (4) distal gonimoblast filaments that are narrowly rectilinear, the terminal spores apparently only rounding up when shed; and (5) tetrasporangia scattered through the surface layers of non-nemathelial branch tips and associated by undivided paraphyses cells (Kraft 1977).

Molecular evidence

Pinnatiphycus clearly belongs in the Dicranemataceae on the basis of its vegetative and reproductive morphology discussed above, and this placement is robustly supported by its *rbcL* gene sequence (Fig. 20). Taxa from the families Acrotylaceae and Mychodeaceae were included in the analysis, as these families, as well as the Mychodeophyllaceae and Cubiculosporaceae (for which *rbcL* gene sequences were not available), have been shown, on the basis of small-subunit rDNA (SSU) gene sequences, to be related to the Dicranemataceae, although the relationships among the families are not resolved (Saunders *et al.* 2004). *rbcL* sequences from taxa in the Sarcodiaceae were also included, as Norris (1987) had proposed that the Dicranemataceae be submerged into the Sarcodiaceae, an idea first refuted by Liao *et al.* (1993) on the basis of both nonfibrillar cell wall biochemistry and reproductive morphology, and later by Saunders *et al.* (2004) on the basis of SSU gene sequences. A close relationship of taxa in Sarcodiaceae and Dicranemataceae is likewise not supported by the *rbcL*-gene analyses. The Dicranemataceae is only weakly supported as a family distinct from the Mychodeaceae/Acrotylaceae clade in this analysis, but the inclusion of other taxa in the family, namely *Peltasta* and *Reptataxis*, likely would strengthen support for it, as well as elucidate interfamily relationships among the genera.

Biogeographical considerations

The description of the new genus and species extends the distribution of the family Dicranemataceae from primarily the southern and southwestern coasts of Australia to the central southwestern Pacific. Current checklists, field guides and floras for Fiji (N'Yeurt *et al.* 1996; N'Yeurt 2001; Littler & Littler 2003; South & Skelton 2003) and New Caledonia (Garrigue & Tsuda 1988; Millar & Payri, in press) do not report any members of the Dicranemataceae, and none have been listed east of Fiji in the South Pacific, although an apparently new member of this family with a *Reptataxis* habit but *Tylotus*-type 'cellular' medulla, hemispherical carpospophytes and highly nemathelial tetrasporangia is known from low-intertidal and shallow-subtidal habitats in the Hawaiian Islands (G.T. Kraft, personal communication from, e.g., BISH, IA 130, IA

4270, IA 14694) and *Dicranema rosaliae* has been reported from Micronesia (Lobban & Tsuda 2003) despite the virtual removal of that species from the genus and family by Kraft (1977, p. 235).

The superficial resemblance of *Pinnatiphycus menouana* to *Callophycus densus* (Sonder) Kraft (family Solieriaceae) might have led to misidentifications in the past, but the latter species is thinner, lacks long cylindrical lateral branchlets, has a whitish cast (due to the presence of bromine) and an apparent midrib (Kraft 1984, p. 58), and could not be confused to the trained eye. Also, it has carpogonial branches oriented sideways to the surface, cystocarps borne on the main axis, tetrasporangia throughout the thallus cortex, and is dioecious. As both genera occur in Fiji and New Caledonia, previous records of *Callophycus* from these localities should be re-examined to confirm that they are not *P. menouana*. *Pinnatiphycus* may be more widespread in the tropical Pacific and elsewhere, but its predilection for very deep water, or turbid high-current habitats with low light intensity, may have prevented it from being collected in the past.

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