Eur. J. Phycol. (2003), **38**: 143–151.

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Nitophyllum hommersandii sp. nov. (Delesseriaceae, Rhodophyta) from Taiwan

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(*Received 15 June 2002; accepted 17 December 2002*)

A new member of the Delesseriaceae (Ceramiales, Rhodophyta) is described from Kenting National Park, southern Taiwan. On the basis of comparative vegetative and reproductive morphology, and phylogenetic analysis inferred from chloroplast-encoded *rbc*L sequences, we conclude that it belongs in the genus *Nitophyllum*, tribe Nitophylleae, subfamily Nitophylloideae. The new taxon shares the following features with the type species of *Nitophyllum*, *N. punctatum* (Stackhouse) Greville: the absence of macro- and microscopic veins; diffuse growth by marginal and intercalary multinucleate meristematic cells; discoid chloroplasts; mature procarps scattered along the thallus margin on both sides, each consisting of a supporting cell bearing a straight, 4-celled carpogonial branch flanked laterally by a cover cell and one sterile group, two connecting cells cut off from the fertilized carpogonium, an auxiliary cell cut off apically from the supporting cell and diploidized by one of two connecting cells; gonimoblasts irregularly subdichotomously branched, bearing terminal carposporangia; a persistent, distinct and uninucleate basal gonimoblast cell; cell fusions entirely absent but broadened pit plugs among gonimoblast cells; and vegetative cells flanking basal gonimoblast cell in the floor of the mature cystocarp dividing to form a rudimentary dome-shaped mount. The new species is distinguished from other members of the genus by the formation of extensive tangled mats of prostrate and overlapping decumbent blades, rhizoidal haptera borne underneath the prostrate blades and marginal rhizoidal filaments along the thallus margin; formation of multiple secondary pit connections among vegetative cells; and spermatangial sori small, numerous and scattered over the fertile blades. We herein describe *Nitophyllum hommersandii* sp. nov. as a new species in the genus. A comprehensive revisionary study of *Nitophyllum* and of the generic concepts in the Nitophylleae is recommended.

Key words: *Calonitophyllum*, Delesseriaceae, Gulf of Mexico, marine algae, Nitophylleae, *Nitophyllum hommersandii* sp. nov., phylogeny, *rbc*L, Rhodophyta, systematics, Taiwan

Introduction

In their phylogenetic treatment of the Delesseriaceae, Lin *et al.* (2001*a*) emended the subfamily Nitophylloideae of Kylin (1924, 1956) by restricting it to two assemblages equivalent to tribes: the Nitophylleae and Martensieae. The Nitophylleae consists of six non-parasitic genera: *Nitophyllum* Greville (1830), *Polyneuropsis* Wynne, McBride *et* West (1973), *Calonitophyllum* Aregood (1975), *Valeriemaya* Millar *et* Wynne (1992), *Papenfussia* Kylin (1938) and *Radicilingua* Papenfuss 1956 (Lin *et al.*, 2001*a*). The Nitophylleae is characterized by vegetative growth via a marginal meristem and intercalary cell divisions; a thallus lacking lattice-work or perforations; procarps scattered along the margin of young blades on both sides of the thallus and consisting of one lateral sterile group and one straight 4-celled carpogonial branch flanked laterally by the sterile group and associated or not with one group of cover cells; lack of a massive fusion cell, and cell fusions entirely absent (Maggs & Hommersand, 1993; Lin *et al.*, 2001*a*, *c*). The Nitophylleae is paraphyletic, forming a grade with the Martensieae in a global phylogeny of the family based on both chloroplast-encoded *rbc*L and nuclear LSU rDNA gene sequence analyses (Lin *et al.*, 2001*a*).

The genus *Nitophyllum* includes 19 to 30 species with a worldwide distribution ranging from temperate to tropical oceans (Wynne, 1997; for all currently accepted names see Guiry & Nic Dhonncha, 2002). An undescribed membranous and delicate species of *Nitophyllum* was first collected in Kenting National Park, southern Taiwan, during the summer of 2002. In this paper we describe the species as new on the basis of its vegetative and reproductive morphology, and

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ISSN 0967-0262 print/ISSN 1469-4433 online

provide further evidence of its taxonomic placement as inferred from gene sequence analysis among selected members of the Nitophylloideae.

Materials and methods

Collections were made by either SCUBA or snorkelling. Algal samples for the molecular study were desiccated in silica gel or preserved in 95% alcohol. Voucher specimens and materials used in the morphological study were fixed in 10% formalin/seawater, and then stored in 5% formalin/seawater or pressed as herbarium sheets, and deposited in the Herbarium of the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan and the University of Louisiana at Lafayette (LAF), USA. Whole-mount material and hand-sections were stained in 1% aniline blue acidified with 1% HCl and mounted in glycerol or were treated with Wittmann's aceto-iron-haematoxylin-chloral hydrate (Wittmann, 1965) and mounted in 50% Hoyer's mounting medium (Hommersand & Fredericq, 1997; Lin *et al.*, 2001*b*). Photographs of type specimens were taken using a Polaroid DMC Ie digital camera. Microphotographs were taken on an Olympus BX60 microscope with a Polaroid DMC Ie digital camera. Digital images were edited and assembled in plates using PhotoShop v.4.0.

DNA samples were prepared using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) following the instructions of the manufacturer. The gene selected is chloroplast-encoded *rbc*L. The primers and protocols for *rbc*L amplification and automated sequencing used in this study are listed in Lin *et al.* (2001*a*). New sequence data and those first generated in Lin *et al.* (2001*a*) were compiled and aligned with Sequencher (Gene Codes, Ann Arbor, MI) and exported for phylogenetic analysis. Phylogenetic analyses were performed using the Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithms available in the computer program PAUP (v.4.0b10; Swofford, 2002). The *rbc*L alignment initially included 1467 sites, but because information was missing for the 5' ends of many sequences the first 60 sites were excluded from the analyses. Sequences from 13 taxa in the subfamily Nitophylloideae (Table 1) were selected for the analyses; the outgroup consisted of two species in the subfamily Phycodryoideae. For ML the aligned sequences were first analysed with the software Modeltest v.3.0 (Posada & Crandall, 1998) which compared different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fitted the sequence data. The optimal model found was a F81 + G evolutionary model (Kimura 1981 + Gamma distribution). The parameters were as follows: assumed nucleotide frequencies A = 0.3808; C = 0.1784; G = 0.1539; T = 0.2869; substitution rate matrix with A–C substitutions = 1.0, A–G = 0.846, A–T = 1.0, C–G = 1.0, C–T = 0.1625, G–T = 1.0; proportion of sites assumed to be invariable = 0; rates for variable sites assumed to follow a gamma distribution with shape parameter = 1.6368. These values were imported into a ML analysis using the Neighbor Joining (NJ) option in PAUP.

Parsimony heuristic searches consisted of 500 random stepwise additions, MULPARS (but holding only 5 trees at each step) and Tree-Bisection-Reconnection (TBR) swapping algorithm until swapping was complete. The searches were done on each data set under the criterion of equal weights for all substitutions. Consistency (CI) and retention (RI) indices (Kluge & Farris, 1989) were calculated excluding uninformative characters.

Table 1. List of species sequenced in this study and GenBank accession numbers in GenBank. The number after the accession number is the percentage of the gene sequenced

Species	GenBank accession no.	Collection information/references
'*Hemineura*' *cruenta* Harvey	AF257453, 98%	Lin *et al.* (2001*a*)
'*Neomartensia*' *flabelliformis* (Harvey *ex* J. Agardh) Yoshida *et* Mikami	AF257376, 80%	Lin *et al.* (2001*a*)
Calonitophyllum medium (Hoyt) Aregood	AF254166, 97%	Offshore Louisiana, USA; ~55–75 m, dredge, coll. S.-M. Lin & S. Fredericq, 23.viii.98
Martensia elegans Hering	AF257375, 98%	Lin *et al.* (2001*a*)
Martensia martensii (Schmitz) Lin, Fredericq *et* Liao	AF257407, 93%	Lin *et al.* (2001*b*)
Martensia pavonia (C. Agardh) J. Agardh	AF257379, 91%	Lin *et al.* (2001*a*)
Nitophyllum adhaerens Wynne	AF257399, 98%	Flower Garden Banks National Marine Sanctuary, Texas, USA, SCUBA, 21 m; coll. B. Wysor, 15.ii.00
Nitophyllum delicatum Millar	AF257400, 97%	Lin *et al.* (2001*a*)
Nitophyllum hommersandii sp. nov.	AY118270, 96%	Banana Bay, Kenting National Park, S. Taiwan; coll. S.-M. Lin, 19.viii.00
Nitophyllum punctatum (Stackhouse) Greville	AF257402, 97%	Lin *et al.* (2001*a*)
Nitophyllum sp. 1	AF257403, 96%	Galeta, Colon, #1005, Caribbean Sea, Panama; coll. B. Wysor, 21.ix.99
Nitophyllum sp. 2	AF257405, 97%	Lin *et al.* (2001*a*)
Phycodrys riggii Gardner	AF257430, 94%	Lin *et al.* 2001a
Phycodrys rubens (Linnaeus) Batters	AF257429, 95%	Lin *et al.* (2001*a*)
Polyneuropsis stolonifera Wynne, McBride *et* West	AF257439, 93%	Lin *et al.* (2001*a*)

Support for nodes was determined by calculating bootstrap proportion values (Felsenstein, 1985) using MP (5000 bootstrap replicates) and ML methods (100 bootstrap replicates).

Specimens of *Nitophyllum hommersandii* Lin *et* Fredericq, sp. nov. examined morphologically in this study were collected in Kenting National Park, southern Taiwan, from the following sites: (a) Banana Bay (21°55′N; 120°49.93′E), coll. S.-M. Lin, cystocarpic, 1–5 m depth, 19.viii.00; tetrasporic, 1–5 m depth, 10.i.02; spermatangial, 1–5 m depth, 12.iii.02; cystocarpic, 1–5 m depth, 29.iii.02; (b) Sail Rock (21°55′N; 120°49.44′E), coll. S.-M. Lin, tetrasporic, 1–3 m depth, 14.iii.02; cystocarpic, 1–3 m depth, 1.iv.02.

New *rbc*L sequences were generated for *Nitophyllum hommersandii* sp. nov., *N. adhaerens*, *Nitophyllum* sp. 1 and *Calonitophyllum medium* (See Table 1 for GenBank numbers and locality data).

Results

Nitophyllum hommersandii Lin *et* Fredericq, sp. nov. (Figs 1–25)

Thalli constantes aliquantum ex implexis tegetibus laminis prostratis decumbentibusque laminis decumbentibus lobatis sine stipiteque haptero; laminae lobatae usque ad 3 cm longaeque 0.5–6 mm latae, tegetes usque ad 13 cm latae, affixae per substratum uniseriatis rhizoideis marginalibus filamentosis subter laminis prostratis; laminae membranaceae monostromaticae omnino; macroscopicae et microscopicae venae absentes; gametophyti dioecii, sori masculini parvi irregularesque dispersi super laminis fertilibus utrinque strati centralis; procarpia formantia utrinque laminarum fertilium, ubi non fecundata constantia ex cellula sustinenti ferenti rectum 4-cellulare filum carpogoniale circuncinctum lateraliter cellula tecta et unica cellula sterili; duo cellulae connexae factae carpogonio fecundato, cellula auxiliari abscissa cellula sustistenti; diploidea a una cellularum connexarum ante abscissa initia gonimoblasti; fila gonimoblasti irregulariter subdichotoma, ferentia carposporangia terminaliter; cellula basalis carposporophyti parva uninucleataque sine conjunctionibus cellulis intimis gonimoblasti; initium tetrasporangii abscissum e cellulis subsuperficialibus; tetrasporangia disposita in duo series ad maturitatem.

Thalli consisting of extensive tangled mats composed of prostrate and overlapping, decumbent, lobed blades lacking stipe and holdfast; lobed blades each up to 3 cm in length by 0.5–6 mm in width, mats reaching up to 13 cm in width, attached to the substratum by rhizoidal haptera borne underneath the prostrate blades and by marginal rhizoidal filaments along the thallus margin; blades membranous and monostromatic throughout; macro- and microscopic veins absent; gametophytes dioecious; spermatangial sori minute, irregular patches, scattered over fertile blade, formed on both sides of central layer; procarps formed on both sides of fertile blades, when unfertilized consisting of a supporting cell bearing a straight, 4-celled carpogonial branch flanked laterally by a cover cell and one sterile cell; two connecting cells cut off from fertilized carpogonium, auxiliary cell cut off apically from supporting cell, diploidized by one of the two connecting cells before cutting off gonimoblast initials; gonimoblast filaments irregularly subdichotomous, bearing carposporangia terminally; basal gonimoblast cell persisting and uninucleate; vegetative cells flanking basal gonimoblast cell in floor of mature cystocarp dividing to form an elevated dome-shaped mount; cell fusions entirely absent; tetrasporangial initial cut off from subsurface cortical cells, tetrasporangia arranged in two rows at maturity.

ETYMOLOGY: '*hommersandii*' is named in honour of Professor Max Hommersand to recognize his groundbreaking studies in the reproductive morphology and systematics of the Delesseriaceae.

HOLOTYPE: in NMMBA, 4-01-2002-SR-1 (Fig. 1). Isotypes in NMMBA and LAF, 3-14-2002-SR-1 to 3-14-2002-SR-6.

TYPE LOCALITY: Sail Rock, Kenting National Park, southern Taiwan (21°55′N; 120°49.44′E).

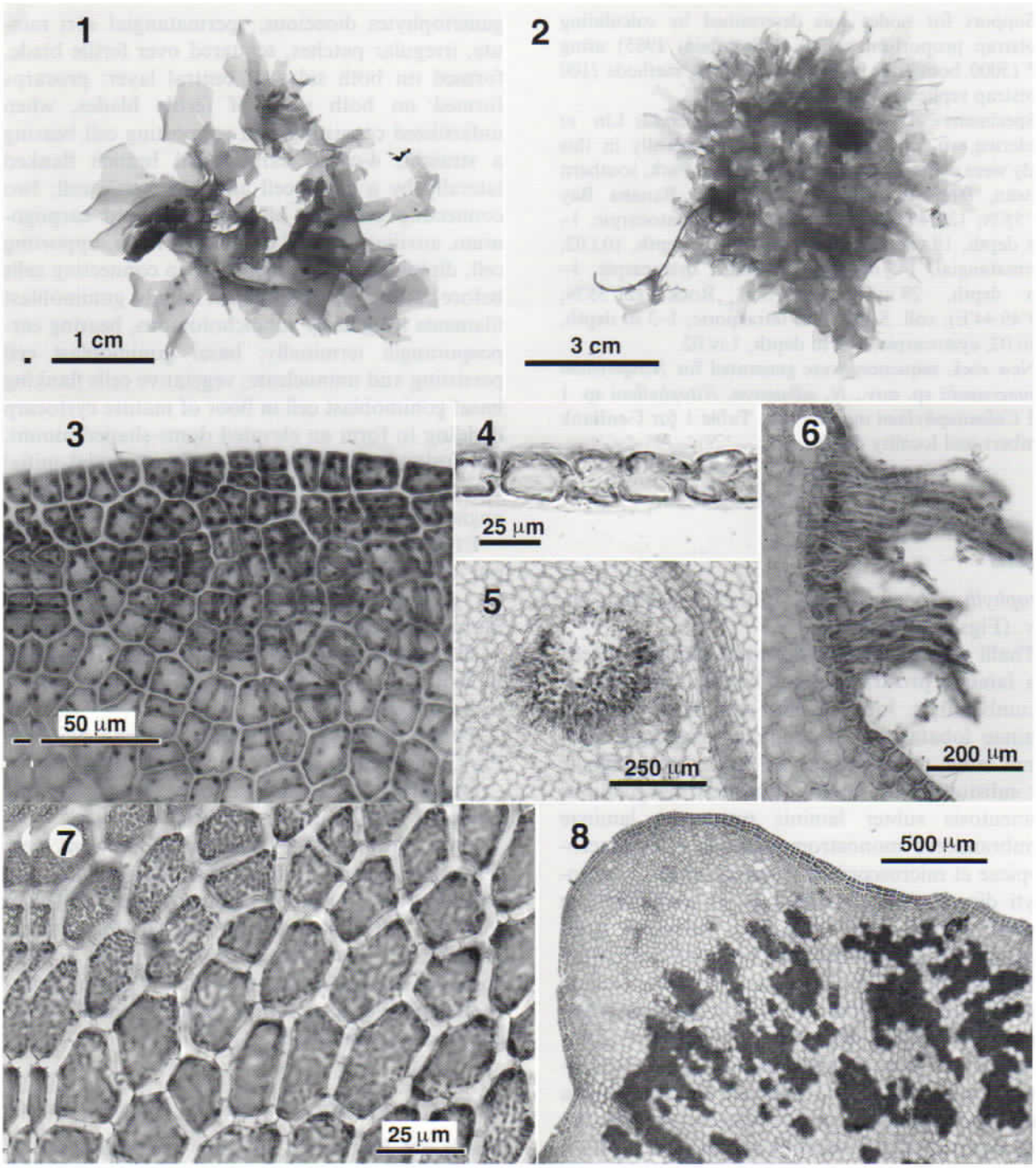
DISTRIBUTION: Known only from Kenting National Park, southern Taiwan.

HABITAT AND SEASONALITY: Collections were seasonally made in March, April, August 2001 and January 2002. Lack of perennial stipes indicates that this taxon may be an annual. Plants were growing at 1–5 m depths on coral reefs, or were epiphytic on the marine red algae *Gelidiopsis repens* (Kützing) Weber-van Bosse and *Drachiella liaoii* Lin, Lewis *et* Fredericq.

Habit and vegetative structure

Thalli are bright red to pink and composed of prostrate and overlapping decumbent lobed blades forming mats 1.5–3 cm high and up to 13 cm wide (Figs 1, 2). Decumbent blades are subdichotomously to irregularly lobed, with the free ends unevenly 0.5–6 mm wide giving most plants highly irregular outlines (Figs 1, 2).

Growth is diffuse by the meristematic activity of multinucleate (Fig. 3) marginal and intercalary cells. Blades are bright red to pink. Blades are membranous and monostromatic throughout except for the reproductive portions, and measure 20–35 μm in thickness (Fig. 4). Margins are otherwise smooth and entire (Fig. 3). Micro- and macroscopic veins are absent throughout. A holdfast or recognizable stipe is absent, and the blades are anchored directly to the substratum by

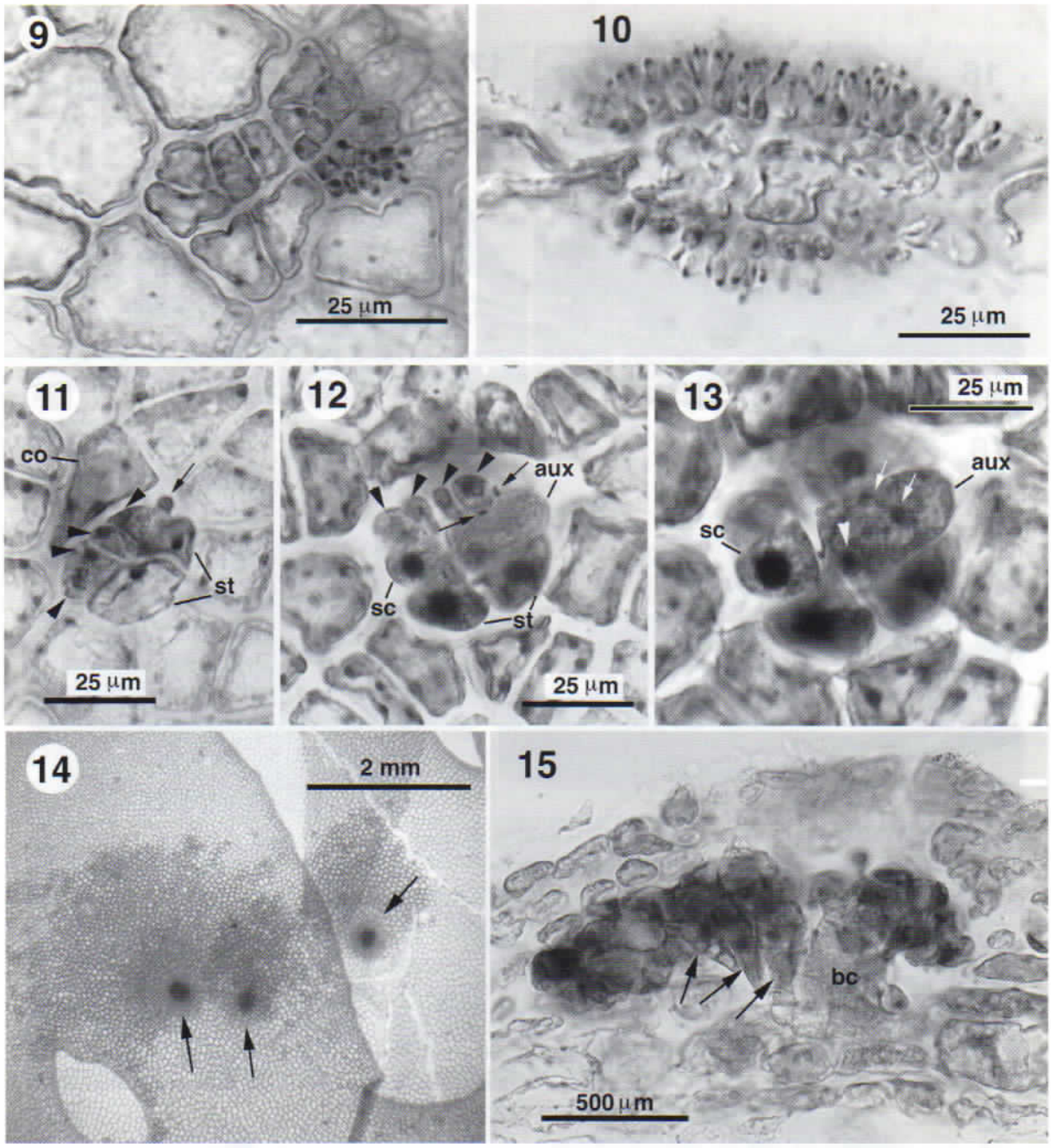


Figs 1–8. *Nitophyllum hommersandii* Lin *et* Fredericq sp. nov. Habit, vegetative and reproductive morphology (Figs 1–7: Sail Rock, Kenting National Park, Fig. 8: Banana Bay, Kenting National Park). Fig. 1. Holotype, cystocarpic specimen. Fig. 2. Syntype, tetrasporangial specimen. Fig. 3. Young blade margin showing marginal and intercalary meristematic region; all cells are multinucleate. Fig. 4. Cross-section through a monostromatic thallus. Fig. 5. One of the anchoring haptera underneath a prostrate blade. Fig. 6. Marginal rhizoidal filaments in an old blade. Fig. 7. Surface view showing multiple pit plugs among contiguous cells, and discoid plastids. Fig. 8. Surface view of mature spermatangial sori scattered over a fertile blade.

uniseriate, multinucleate rhizoidal filaments extending from marginal surface cells or by haptera borne on the undersurface of prostrate blades (Figs 5, 6). Seen from above, there are numerous discoid plastids per surface cell (Fig. 7). Two multinucleate neighbouring cells can share more than one secondary pit plug between them (Fig. 7).

Reproductive structures

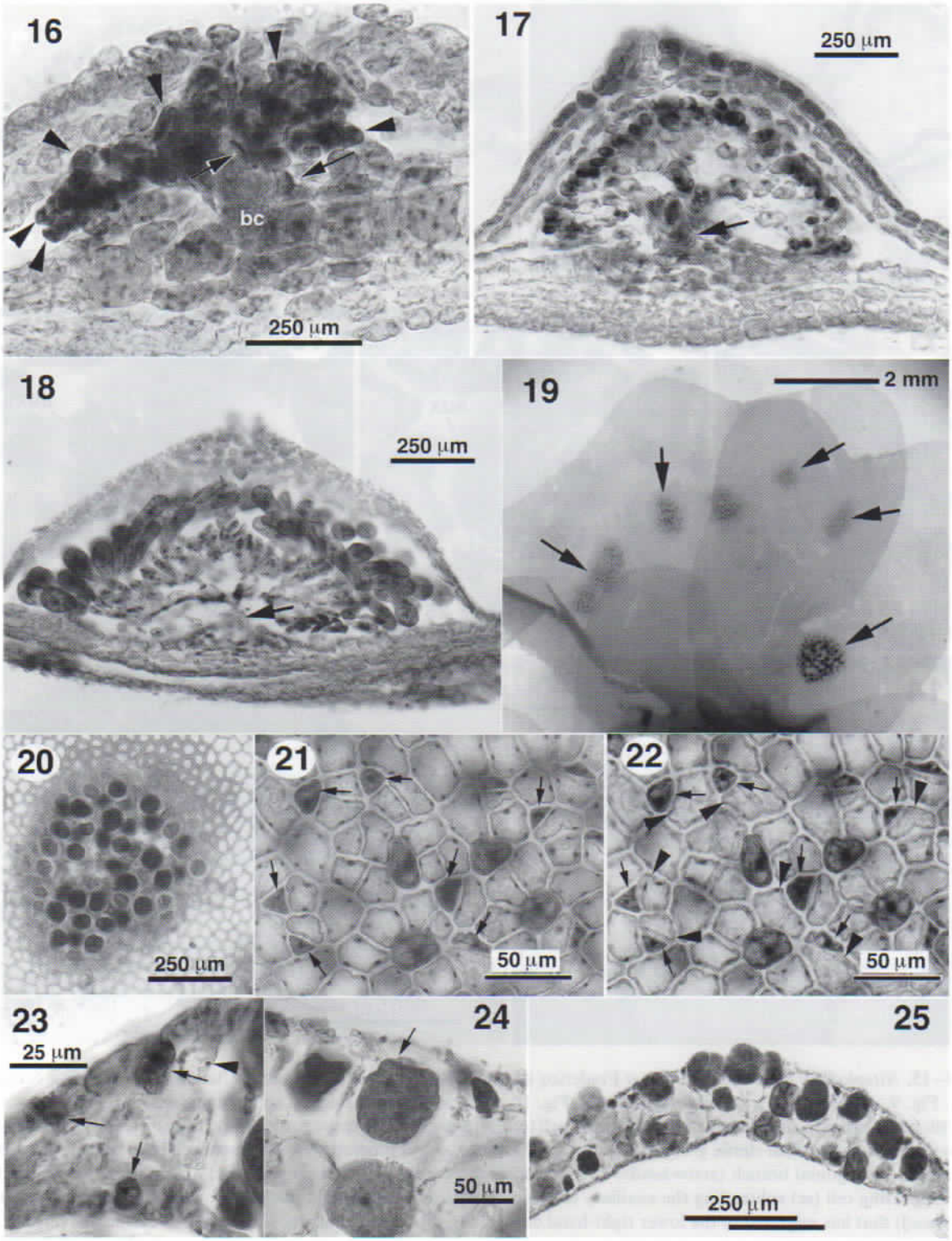
All reproductive structures are scattered over both sides of the fertile blades (Figs 8, 14, 19). Gametophytes are dioecious and isomorphic with the tetrasporophytes. Spermatangial sori are minute and irregularly elliptical, and may coalesce into convoluted patches, 25–600 μm long × 25–



Figs 9–15. *Nitophyllum hommersandii* Lin *et* Fredericq sp. nov. Reproductive morphology (Banana Bay, Kenting National Park). Fig. 9. Close up of a spermatangial sorus. Fig. 10. Cross-section through a spermatangial sorus. Fig. 11. Prefertilization procarp showing an undivided cover cell (co), straight 4-celled carpogonial branch (arrowheads) with trichogyne (arrow), and lateral 2-celled sterile group (st). Fig. 12. Postfertilization procarp with supporting cell (sc) bearing the auxiliary cell (aux), a carpogonial branch (arrowheads) having cut off two connecting cells (arrows) and a 2-celled sterile group. Fig. 13. Supporting cell (sc) subtending the auxiliary cell (aux) containing two diploid nuclei (arrows) and a haploid nucleus (arrowhead) that has migrated to the lower right-hand corner. Fig. 14. Fertile blades with scattered cystocarps (arrows). Fig. 15. Cross-section showing young gonimoblast cells (arrows) subtended by the persisting basal gonimoblast cell (bc).

500 μm wide (Fig. 8). Surface cells become spermatangial mother cells (Fig. 9) that cut off one to three elongate spermatangia 3–5 μm long × 1–2 μm wide (Fig. 10).

Procarps are abundant near the blade margins, and unfertilized procarps consist of a fertile central cell cutting off two pericentral cells, one of which functions as the cover cell while the other becomes the large supporting cell that cuts off laterally a straight 4-celled carpogonial branch and one 2-celled sterile group (Fig. 11). Direct fertilization was not seen in our material. Following presumed fertilization, the fertilized carpogonium cuts off two minute connecting cells and the supporting cell cuts off a large auxiliary cell (Fig. 12). The carpogonial branch degenerates after diploidization of the



Figs 16–25. *Nitophyllum hommersandii* Lin *et* Fredericq sp. nov. Cystocarp and tetrasporangial features (Figs 16–17: Banana Bay, Kenting National Park; Figs 18–25: Sail Rock, Kenting National Park). Fig. 16. Cross-section through a young cystocarp showing the basal gonimoblast cell (bc) bearing gonimoblast filaments terminating in carposporangial initials (arrowheads). Note the broadened pit connections (arrows) between the basal gonimoblast cell and lowest cells of gonimoblast filament. Fig. 17. Cross-section through a young cystocarp showing the uninucleate basal gonimoblast cell (arrow). Fig. 18. Cross-section through a fully mature cystocarp showing the persisting basal gonimoblast cell (arrow). Fig. 19. Tetrasporangial sori (arrows) scattered over the fertile blades. Fig. 20. Surface view of a tetrasporangial sorus. Fig. 21. Surface view of tetrasporangial initials (arrows). Fig. 22. Same as in Fig. 21, but focused on the subsurface plane showing tetrasporangial initials (arrow) cut off from subcortical cells (arrowheads). Fig. 23. Cross-section through a young tetrasporangial sorus showing tetrasporangial initials (arrows) cut off laterally from the subsurface cortical cells (arrowhead). Fig. 24. Cross-section through an immature tetrasporangial sorus showing a 4-nucleate tetrasporangium (arrow) before its division into tetraspores. Fig. 25. Cross-section through a mature tetrasporangial sorus showing tetrasporangia arranged in two rows.

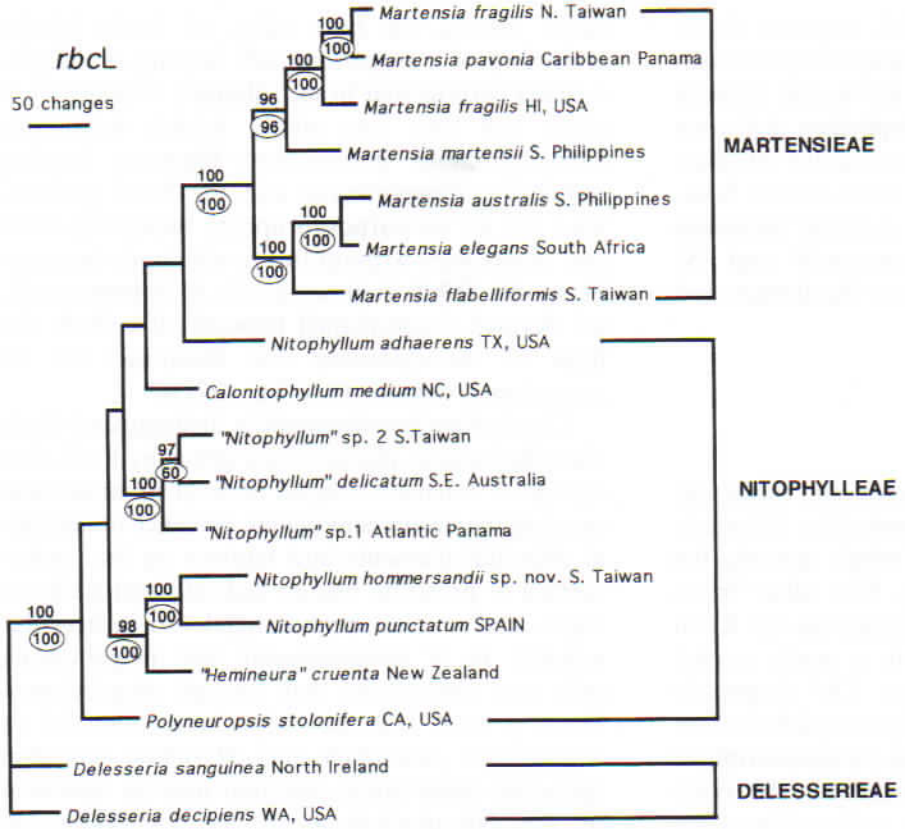


Fig. 26. Maximum likelihood tree for *rbc*L sequences showing the inter- and intrageneric relationships of Nitophylleae using *Phycodrys* spp. as outgroup taxa. Bootstrap proportion values (> 50%) for maximum likelihood (ML; top, 100 replicates) and maximum parsimony (MP; bottom, 5000 replicates) are shown at the nodes.

auxiliary cell; in the auxiliary cell the diploid nucleus divides once and the haploid nucleus migrates to the lower right-hand corner of the auxiliary cell (Fig. 13).

At an early stage of carposporophyte growth the supporting cell functions as a basal cell and remains uninucleate at the base of the carposporophyte (Fig. 15). As gonimoblast development progresses, pit connections linking gonimoblasts to the basal gonimoblast cell broaden (Fig. 16), but there are no cell fusions among gonimoblast cells or between the basal cell and inner gonimoblast cells. Consequently, the gonimoblasts remain connected to a persisting, uninucleate basal gonimoblast cell (Figs 17, 18). Vegetative cells flanking the basal gonimoblast cell in the floor of the mature cystocarp divide to form a rudimentary dome-shaped mount (Fig. 18). Cystocarps are formed on both sides of the blades, reach 750–1000 μm in diameter at maturity, and are hemispherical with a 2- to 3-cell-layered pericarp with a slightly protuberant central ostiole (Figs 17, 18). Pyriform carposporangia, 35–55 μm × 100–125 μm, are formed terminally (Fig. 18).

Tetrasporangial sori are rounded to ovoid in shape (Figs 19, 20), 400–750 μm × 550–900 μm in diameter, and solitary or slightly aggregated. Tetrasporangial initials are cut off laterally from subsurface cortical cells (Figs 21–24). Mature tetrasporangia are tetrahedrally divided, 90–100 μm × 100–115 μm in diameter, mature at an irregular rate, and are arranged on both sides of the central cells (Fig. 25).

Molecular analyses

*Rbc*L sequences of *N. hommersandii* sp. nov. from Kenting National Park, Southern Taiwan (type locality), *N. adhaerens*, and *Calonitophyllum medium* from the Northwestern Gulf of Mexico were newly generated. The final *rbc*L data matrix was restricted to 1407 total sites. The topology of the MP and ML trees was identical; only the inferred ML-based tree reconstructed from the Hierarchical Likelihood Ratio Tests (hLRTs) F81 + G model of sequence evolution is presented (Fig. 26). Parsimony analysis revealed a single most-parsimonious tree (length = 1038, CI = 0.5934, RI = 0.5654, and 355 informative characters).

The tribe Nitophylleae, using the Phycodryeae as the outgroup, is shown to be paraphyletic (Fig. 26) forming a grade leading to the Martensieae. The Martensieae is a terminal monophyletic clade with full bootstrap support. There are two strongly supported groups within *Nitophyllum*, one consisting of the undescribed species from southern Taiwan along with the type species, *N. punctatum*, and '*Hemineura*' *cruenta* (99/90% bootstrap support), and the other consisting of *Nitophyllum delicatum* from S.E. Australia and two undescribed species from Caribbean Panama (96/95% boot-

strap support). Interspecific *rbc*L sequence divergence among species of the *Nitophyllum punctatum* clade varied between 8.4% and 9.3%, and between 2.4% and 4.3% within the *Nitophyllum delicatum* clade. The positions of *N. adhaerens* and *C. medium* from the NW Gulf of Mexico received low bootstrap support and are poorly resolved. Sequence divergence between *N. hommersandii* and *N. adhaerens* is 11.3% and between the former and *C. medium* is 10.3%.

Discussion

On the basis of both morphological and molecular evidence, Lin *et al.* (2001*a*) emended the Nitophylloideae to contain only two tribes, namely the Nitophylleae and Martensieae. The other tribes formerly placed in the Nitophylloideae by Kylin (1924, 1956) were transferred to a newly erected subfamily, the Phycodryoideae. The diagnostic character for separating the Phycodryoideae from the Nitophylloideae pertains to carposporophyte development. In the Phycodryoideae, the post-fertilization fusion cell is large and multinucleate due to the fusion of the auxiliary cell with inner gonimoblast cells and neighbouring gametophytic cells, and the pit connections linking individual gonimoblast cells degenerate. In contrast, in the Nitophylloideae, a fusion cell or fusion cell products are entirely lacking, the basal gonimoblast cell remains distinct and uninucleate, and the pit connection linking each gonimoblast cell persists and expands in size (Lin *et al.*, 2001*a*, 2002).

The Nitophylleae has already been shown to be paraphyletic based on both *rbc*L and LSU rDNA sequence analyses (Lin *et al.*, 2001*a*). Whereas the post-fertilization development of '*Erythroglossum' undulatissimum* (also known as '*Hemineura' cruenta*, see Lin *et al.*, 2001*a*, p. 892) and *Nitophyllum punctatum* have been studied in detail (Hommersand, unpublished data), it is poorly studied in most genera of the Nitophylleae (Kylin, 1924, 1956; Newton, 1931; Maggs & Hommersand, 1993). Post-fertilization development of '*E'. undulatissimum* and *N. punctatum* is shown to be very similar to that of *N. hommersandii* described here. Since representative species of two other genera in the Nitophylleae, *Papenfussia* and *Radicilingua*, have not been sequenced, and since most species in this tribe are in need of detailed morphological study, we provisionally view the Nitophylleae as paraphyletic and *Nitophyllum* as polyphyletic.

Nitophyllum hommersandii shares with the type of the genus, *N. punctatum* (Maggs & Hommersand, 1993): an absence of macro- and microscopic veins; a diffuse growth by marginal and intercalary meristematic cells; a monostromatic thallus; procarps formed on both sides of fertile blades consisting of a supporting cell bearing a straight, 4-celled carpogonial branch flanked laterally by a cover cell and one sterile group; irregularly subdichotomous gonimoblast filaments bearing terminal carposporangia; and the basal gonimoblast cell of the carposporophyte remaining small and uninucleate without fusing with inner gonimoblast cells. While rudimentary in *N. hommersandii*, an elevated dome-shaped network of cells in the floor of the cystocarp was illustrated for *N. punctatum* (Newton, 1931: fig. 197D).

Nitophyllum hommersandii is distinguished from the other species placed in the genus by thalli that consist of extensive tangled mats of prostrate and overlapping decumbent blades, presence of marginal rhizoidal filaments and haptera on the undersurface of prostrate blades, lack of basal stalks or stipes, and minute spermatangial sori in irregular patches. In *N. hommersandii*, two neighbouring cells may share more than one pit plug between them, a trait that has also been observed in *Nitophyllum punctatum* and *Martensia martensii* (personal observation) and that may be restricted to the Nitophylloideae in the Delesseriaceae. Similar linkage of secondary pit plugs is also found in the Phyllophoraceae (personal observation, Maggs *et al.*, 1992) but it has not been reported for other red algae. Among the described species of *Nitophyllum*, only *N. adhaerens* (Wynne, 1997) from the Caribbean and Bermuda shows some morphological similarities to *N. hommersandii* by its adherent habit and small thallus. However, specimens of *N. adhaerens* are no more than 1.5 cm high, whereas thalli of *N. hommersandii* can reach 3 cm. Moreover, the male and tetrasporangial reproductive structures are scattered over the entire fertile blade in *N. hommersandii* (Figs 2, 9, 19) whereas those in *N. adhaerens* are restricted to the margins of the fertile blades (Wynne, 1997: figs 3–5). The other species placed in *Nitophyllum* are much larger and may or may not have a distinct stipe (see Wynne, 1997; Guiry & Nic Dhonncha, 2002). The two undescribed species of *Nitophyllum* from Caribbean Panama (Table 1, Fig. 26) will be critically compared with material of *Nitophyllum delicatum* from Australia and described as new elsewhere.

Due to similar-looking entangled clumps and prostrate habit, specimens of *Nitophyllum hommersandii* can easily be misidentified in the field as *Drachiella liaoii* (Lin *et al.*, 2002), a member of the Delesseriaceae only reported from Taiwan and the Philippines, and '*Acrosorium venulosum*' (Zanardini) Kylin (1924), a taxon that occurs in the Indo-Pacific Ocean (Silva *et al.*, 1996). However, *Nitophyllum hommersandii* can be distinguished from '*A. venulosum*' (Cryptopleureae) by the lack

of the microscopic veins that characterize the latter, and from the polystromatic *D. liaoii* (Schizoserideae) by its monostromatic thallus.

It is clear that an in-depth generic revision of the Nitophylleae is called for. It is too early to tell, however, whether *N. adhaerens* and the clade centred around *N. delicatum* should represent separate, new genera or whether these taxa will need to be merged with existing genera of the Nitophylleae. Even so, on the basis of the morphological and molecular results presented here, we feel confident that, following the type method (Silva, 1952), our new species fits the generic concept of *Nitophyllum* (Maggs & Hommersand, 1993).

This is the first-named species in the genus *Nitophyllum* from Taiwan and the first report of the monotypic genus *Calonitophyllum* outside the Carolinas (Schneider & Searles, 1991).

Acknowledgements

This study was supported by the National Museum of Marine Biology and a grant from the Kenting National Park, Minister of Interior SML. S.M.L. thanks I.-S. Chen at the National Museum of Marine Biology & Aquarium (NMMBA) for sharing equipment and Y.-M. Ju for assisting with the DNA sequencing of the new taxon. S.M.L. would also like to thank the Director of the Museum, Dr L.-S. Fang, for his support and encouragement during this study. Part of this research was also funded by NSF grant DEB9903900, DOE grant DEFG02-97ER12220, and NURC-NOAA grant NA96RU-0260 to S.F. Special thanks go to B. Wysor for material of *N. adhaerens* and *Nitophyllum* spp., and E. Hickerson for facilitating collecting in the Flower Garden Banks Marine Sanctuary. We thank Dr Hommersand for sharing his vast knowledge of the Delesseriaceae and Nitophylleae with us.

References

Aregood, C.C. (1975). A study of the red alga *Calonitophyllum medium* (Hoyt) comb. nov. [= *Hymenena media* (Hoyt) Taylor]. *Br. Phycol. J.*, **10**: 347–362.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783–791.

Greville, R.K. (1830). *Algae Britannicae*, Edinburgh.

Guiry, M.D. & Nic Dhonncha, E. (2002). Algaebase, World Wide Web electronic publication www.algaebase.com. (14 May 2002 search).

Hommersand, M.H. & Fredericq, S. (1997). Characterization of *Myriogramme livida*, Myriogrammeae trib. nov. (Delesseriaceae, Rhodophyta). *J. Phycol.*, **33**: 106–121.

Kluge, A.G. & Farris, J.S. (1989). Quantitative phyletics and the evolution of anurans. *Syst. Zool.*, **18**: 1–32.

Kylin, H. (1924). Studien über die Delesseriaceen. *Lunds Univ. Årsskr. N.F. Avd. 2*, **20(6)**: 111 pp.

Kylin, H. (1938). Verzeichnis einiger Rhodophyceen von Sudafrica. *Lunds Univ. Årsskr. N.F. Avd. 2*, **34(8)**: 1–26.

Kylin, H. (1956). *Die Gattungen der Rhodophyceen*. CWK Gleerups Forlag, Lund.

Lin, S.-M., Fredericq, S. & Hommersand, M.H. (2001*a*). Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbc*L sequences, including the Phycodryoideae subfam. nov. *J. Phycol.*, **35**: 881–899.

Lin, S.-M., Hommersand, M.H. & Kraft, G.T. (2001*b*). Characterization of *Hemineura frondosa* and the Hemineureae trib. nov. (Delesseriaceae, Rhodophyta) from southern Australia. *Phycologia*, **40**: 135–146.

Lin, S.-M., Fredericq, S. & Liao, L.M. (2001*c*). Neotypification and taxonomic status of *Opephyllum martensii* Schmitz in Schmitz *et* Hauptfleisch (Delesseriaceae, Rhodophyta) from Zamboanga, southern Philippines. *Bot. Mar.*, **44**: 589–595.

Lin, S.-M., Lewis, J.E. & Fredericq, S. (2002). *Drachiella liaoii* sp. nov., a new member of the Schizoserideae (Delesseriaceae, Rhodophyta) from Taiwan and the Philippines. *Eur. J. Phycol.*, **37**: 93–102.

Maggs, C.A. & Hommersand, M.H. (1993). *Seaweeds of the British Isles*. vol. I. *Rhodophyta:* part 3A, *Ceramiales*, The Natural History Museum, London, UK.

Maggs, C.A., Douglas, S.E., Fenety, J. & Bird, C.J. (1992). A molecular and morphological analysis of *Gymnogongrus devoniensis* (Rhodophyta) complex in the North Atlantic. *J. Phycol.*, **28**: 214–232.

Millar, A.J.K. & Wynne, M.J. (1992). *Valeriemaya* gen. nov. (Rhodophyta), with discussion of apical organizations within the Delesseriaceae. *Br. Phycol. J.*, **27**: 131–143.

Newton, L. (1931). *A Handbook of the British Seaweeds*. British Museum (Natural History), London.

Papenfuss, G.F. (1956). On the nomenclature of some Delesseriaceae. *Taxon*, **5**: 158–162.

Posada, D. & Crandall, K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**: 817–818.

Silva, P.C. (1952). A review of nomenclatural conservation in the algae from the point of view of the type method. *Univ. Calif. Publ. Bot.*, **25**: 241–323.

Silva, P.C., Basson, P.W. & Moe, R.L. (1996). *Catalogue of the Benthic Marine Algae of the Indian Ocean*, University of California Press, Berkeley, USA.

Swofford, D.L. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods)*. Version 4.0*, Sinauer Associates, Sunderland, MA.

Wittmann, W. (1965). Aceto-iron-haemotoxylin-chloral hydrate for chromosome staining. *Stain Technol.*, **40**: 161–164.

Wynne, M.J. (1997). *Nitophyllum adhaerens* sp. nov. (Delesseriaceae, Rhodophyta) from the Caribbean and Bermuda. *Cryptog. Algol.*, **18**: 211–221.

Wynne, M.J., Mcbride, D.L. & West, J.A. (1973). *Polyneuropsis stolonifera* gen et sp. nov. (Delesseriaceae, Rhodophyta) from the Pacific coast of North America. *Syesis*, **6**: 243–253.