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Two new species of *Martensia* (Delesseriaceae, Rhodophyta) from Kenting National Park, southern Taiwan

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Two new species of *Martensia* (tribe Martensieae, subfamily Nitophylloideae) are described from Kenting National Park, southern Taiwan. *Martensia lewisiae* sp. nov. can be separated from all other species of *Martensia* by the formation of extensive mats from a creeping system of prostrate and overlapping blades attached by basal and marginal haptera, and by the formation of a weakly developed network only in mature reproductive thalli. *Martensia formosana* sp. nov. is fan-shaped, consisting of one to three flabellate blades attached by a few small discoid holdfasts, with a network consisting of a single continuous band that extends over 60–80% of the blade. It is distinguished by the presence of tetrasporangial sori on primary longitudinal lamellae and bladelets (secondary lamellae) borne on the cross-connecting strands. As is typical for *Martensia*, growth in both species is diffuse, initiated by the meristematic activity of multinucleate marginal and intercalary cells. A network is initiated from a row of transformed marginal cells, each of which cuts off an apical initial that divides to form the longitudinal lamellae and reform the margin of the blade. Cross-connecting strands develop unidirectionally (*M. lewisiae*) or bidirectionally (*M. formosana*) from the primary lamellae. Spermatangial sori are borne on both sides of the blade (*M. lewisiae*) or on the longitudinal lamellae (*M. formosana*). Procarps and cystocarps are formed along the margins of the blade and longitudinal lamellae (*M. lewisiae*) or in the network at the intersections of longitudinal lamellae and cross-connecting strands (*M. formosana*). Postfertilization fusion cells are absent, and the primary pit connections broaden between gonimoblast cells, which remain distinct and uninucleate. Tetrasporangial sori are borne on membranous parts of the blade and rarely on the network (*M. lewisiae*) or on primary lamellae and secondary bladelets within the network (*M. formosana*). Tetrasporangial initials are multinucleate, transformed from cells in monostromatic portions of blades or lamellae. Two well-supported clades were identified in *rbc*L analyses, one containing *M. formosana* and the type species of *Martensia*, *M. elegans*, and the other containing *M. lewisii* and a group of species that cluster with *M. fragilis*. Species richness in *Martensia* is much greater in Taiwan than previously thought, and the same may be true for other regions of the world.

INTRODUCTION

The *Martensia* group, originally proposed by Kylin (1956) to contain *Martensia* Hering (Hering 1841) and *Opephyllum* Schmitz (Schmitz & Hauptfleisch 1897), has been formally recognized as the tribe Martensieae (Wynne 2001). Lin *et al.* (2001a) placed the Martensieae together with the Nitophylleae in the emended subfamily Nitophylloideae on the basis that the procarp consists of a four-celled carpogonial branch flanked on one side by a single lateral sterile group and on the other side by a group of cover cells. Like the Nitophylleae, the Martensieae possesses membranous thalli lacking nerves or veins and a carposporophyte in which the gonimoblast cells remain unfused, linked by broadened primary pit connections. It differs from the Nitophylleae by having the potential to form perforations or networks, especially in reproductive material. The thallus of *Martensia* possesses a more or less regular network, and that of *Opephyllum* is regularly perforated by small circular holes (Kylin 1956).

Agardh (1863) placed *Martensia* in the family Rhodomelaceae (as Rhodomeleae) and recognized six species: *M. flabelliformis* Harvey *ex* J. Agardh (Agardh 1863) from the Friendly Islands (Tonga), *M. australis* Harvey (Harvey 1855) from Western Australia, *M. elegans* Hering (Hering 1841) from eastern South Africa, *M. fragilis* Harvey (Harvey 1854) from Ceylon (present-day Sri Lanka), *M. denticulata* Harvey (Harvey 1855) from Western Australia and *M. pavonia* (J. Agardh) J. Agardh (Agardh 1863) from St Croix, Virgin Islands. Two species were added later: *M. speciosa* Zanardini (Zanardini 1874) from Lord Howe Island and *M. indica* Krishnamurthy & Thomas (Krishnamurthy & Thomas 1977) from Tamil Nadu, India. Lin *et al.* (2001b) transferred *O. martensii* Schmitz to *Martensia* as *M. martensii* (Schmitz) Lin, Fredericq & Liao, and Yoshida & Mikami (1996) established the monotypic genus *Neomartensia* Yoshida & Mikami for *M. flabelliformis*, primarily on the basis that the carposporangia are formed in short chains instead of being terminal. We do not recognize *Neomartensia* in this paper. Agardh (1863) divided *Martensia* into two sections: Hemitrema, in which the network is composed of a single zone within the thallus margin, and Mesotrema, in which the network consists of one to several zones separated by solid membranes. Most workers have not followed Agardh in this treatment. Millar (1990) investigated the lectotype specimens of *M. fragilis*, *M. denticulata* and *M. pavonia* and concluded that all three were similar in stature and habit and could produce either single-banded or multiple-banded networks with smooth or denticulate margins. In all three, the tetrasporangia are borne in both the network and membranous parts of the

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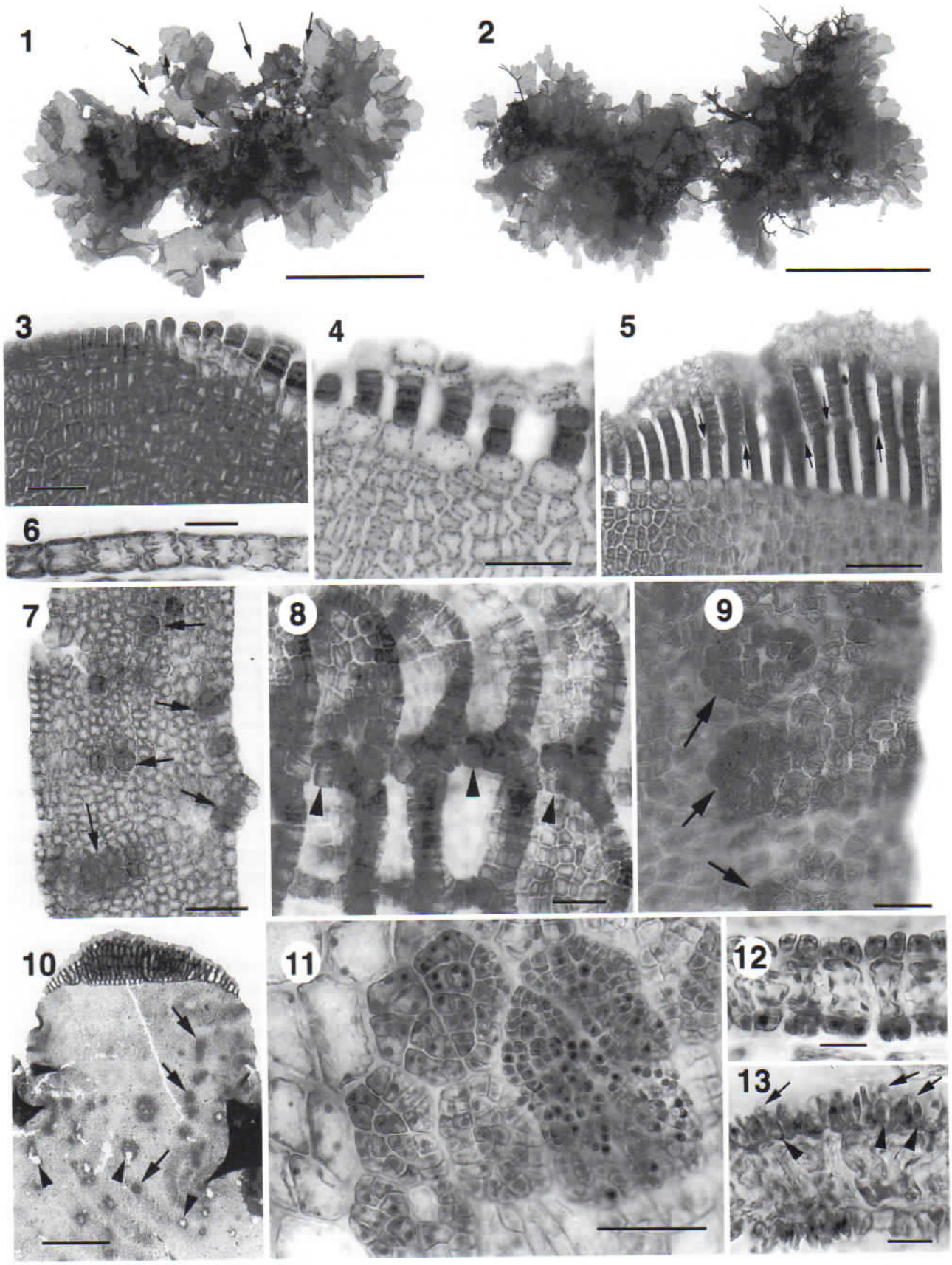

Figs 1–13. *Martensia lewisiae*. Habit and development of vegetative and male structures.
Fig. 1. Holotype; female plant with cystocarps (arrows). Bar = 3 cm.
Fig. 2. Isotype; tetrasporophytic plant. Bar = 3 cm.
Fig. 3. Early stage of network formation showing the initiation of cell rows at the margin. Bar = 50 μm.

Table 1. List of species used in *rbc*L analysis and accession numbers in GenBank. The number after the accession number is the percentage of the gene sequenced.

Species	Collection information/references	GenBank accession number
Calonitophyllum medium (Hoyt) Aregood	Offshore Louisiana, USA (Lin & Fredericq 2003)	AF254166, 97%
"Hemineura" cruenta Harvey	Dl, Vailavae, New Zealand (Lin *et al.* 2001a)	AF257453, 98%
Martensia australis Harvey	Little Santa Cruz Is., Zamboanga City, Philippines (Lin *et al.* 2001b)	AF257374, 94%
Martensia elegans Hering	Palm Beach, Natal Province, South Africa (Lin *et al.* 2001a)	AF257375, 98%
Martensia flabelliformis Harvey *ex* J. Agardh	Wan-Lee-Dong, Kenting National Park, Taiwan (*S.-M. Lin*, 20 Dec. 2001) (this study)	AY253665, 98%
Martensia fragilis Harvey	Little Santa Cruz Island, Zamboanga City, Philippines (Lin *et al.* 2001b)	AF257382, 99%
Martensia "fragilis" Harvey	Chinaman's Hat, Kameohe Bay, Oahu, Hawaii, USA (Lin *et al.* 2001b)	AF257377, 85%
Martensia formosana sp. nov.	Sail Rock, Kenting National Park (*S.-M. Lin*, 2 Oct. 2001) (this study)	AY253663, 97%
Martensia formosana sp. nov.	Wan-Lee-Dong, Kenting National Park (*S.-M. Lin*, 20 Dec. 2001) (this study)	AY253664, 98%
Martensia lewisiae sp. nov.	White Sand Bay, Kenting National Park (*J. Huang*, 13 Feb. 2001) (this study)	AY253662, 96%
Martensia lewisiae sp. nov.	Banana Bay, Kenting National Park (*S.-M. Lin*, 14 Mar. 2001) (this study)	AY253661, 96%
Martensia martensii (Schmitz) Lin, Fredericq & Liao	La Vista Del Mar, Upper Calarian, Zamboanga, Philippines (Lin *et al.* 2001b)	AF257407, 93%
Martensia pavonia (C. Agardh) J. Agardh	Cayos Zapatilla, Bocas del Toro, Caribbean Sea, Panama (Lin *et al.* 2001a)	AF257379, 91%
Nitophyllum delicatum Millar	Jervis Bay, New South Wales, Australia (Lin *et al.* 2001a)	AF257400, 97%
Nitophyllum hommersandii Lin & Fredericq	Banana Bay, Kenting National Park, Taiwan (Lin & Fredericq 2003)	AY118270, 96%
Nitophyllum punctatum (Stackhouse) Greville	Asturias, Spain (Lin *et al.* 2001a)	AF257402, 97%
Phycodrys ovifolia (Kützing) Wynne	Isla Mancerra, Bahia coral, Province Valdivia, Chile (Lin *et al.* 2001a)	AF257423, 94%
Phycodrys rubens (Linnaeus) Batters	West Angle Bay, Pembrokeshire, Wales, UK (Lin *et al.* 2001a)	AF257429, 95%
Polyneuropsis stolonifera Wynne, McBride & West	North Jenner Beach, Sonoma Co., CA, USA (Lin *et al.* 2001a)	AF257439, 93%

blade. Accordingly, he reduced *M. denticulata* and *M. pavonia* to synonymy under *M. fragilis*. If all the changes are accepted (except Yoshida & Mikami 1996), seven species of *Martensia* are currently recognized.

In this paper, we describe two new species of *Martensia* from Kenting National Park, southern Taiwan, based on their vegetative and reproductive morphology. Further evidence of their taxonomic placement in *Martensia* is inferred from chloroplast-encoded *rbc*L sequence analysis of selected members of the Nitophylloideae.

MATERIAL AND METHODS

Collections were made by either scuba diving or snorkelling. 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 transferred to 5% formalin–seawater or pressed on herbarium sheets, and deposited in the Herbarium of the National Museum of Marine Biology and Aquarium (NMMA), Taiwan, and the University of Louisiana at Lafay-

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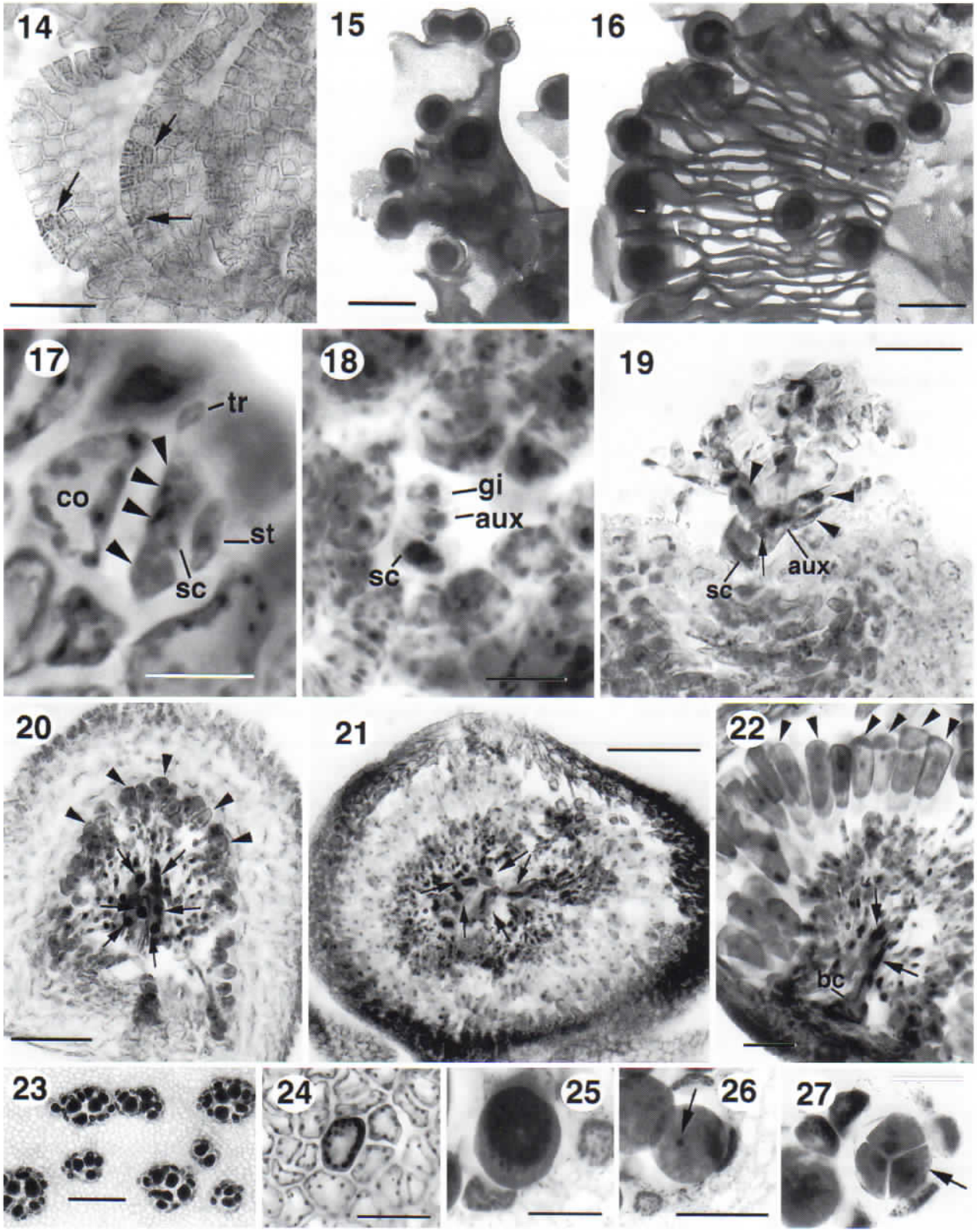
Fig. 4. Elongation of the cell rows by intercalary divisions and the reformation of the membranous margin. Note the enlarged basal cells and spaces between the darkly staining cell rows. Bar = 50 μm.
Fig. 5. Later stage of network formation showing the unilateral initiation (arrows) of cross-connecting filaments and their attachment to the adjacent lamellae. A membranous margin has reformed to the outside with the formation of numerous secondary pit connections. Bar = 100 μm.
Fig. 6. Cross-section of a membranous part of the monostromatic thallus. Bar = 25 μm.
Fig. 7. A single lamella from the network seen in surface view showing some attachment points (arrows) of cross-connecting strands. Bar = 100 μm.
Fig. 8. Oblique view of a fully developed network showing longitudinal lamellae and cross-connecting strands (arrowheads). Bar = 50 μm.
Fig. 9. Margin of prostrate part of thallus showing discoid haptera seen from the lower surface (arrows). Bar = 50 μm.
Fig. 10. Part of a male plant showing spermatangial sori (arrows) situated in the blade portion of the thallus. Note the pores (arrowheads) that are left after spermatangial release. Bar = 500 μm.
Fig. 11. Surface view of a spermatangial sorus. Bar = 50 μm.
Fig. 12. Cross-section of immature spermatangial sorus showing uninucleate spermatangial parent cells. Bar = 10 μm.
Fig. 13. Cross-section through mature spermatangial sorus showing spermatangial parent cells (arrowheads) and uninucleate spermatangia (arrows). Bar = 10 μm.



Figs 14–27. *Martensia lewisiae*. Development of tetrasporangial and female reproductive structures.
Fig. 14. Procarps (arrows) along the edges of longitudinal lamellae. Bar = 100 μm.
Fig. 15. Mature cystocarps along the margin of membranous blade. Bar = 1 mm.
Fig. 16. Mature cystocarps inside the network. Bar = 1 mm.
Fig. 17. Procarp showing a supporting cell (sc) bearing a one-celled sterile lateral (st), a four-celled carpogonial branch (arrowheads) and trichogyne (tr) and a lateral cover cell (co). Bar = 25 μm.

ette (LAF), USA. Whole-mount material and hand-sections were stained in 1% aniline blue acidified with 1% HCl and mounted in 40% Karo syrup–water or were treated with Wittmann's aceto-iron-hematoxylin-chloral hydrate (Wittmann 1965) and mounted in 50% Hoyer's mounting medium (Hommersand & Fredericq 1997; Lin *et al.* 2001b). Photographs of type specimens were taken using a DMC Ie digital camera (Polaroid, Pasadena, CA, USA). Microphotographs were taken on an Olympus BX60 microscope (Olympus America, Melville, NY, USA) with a Polaroid DMC Ie digital camera.

DNA samples were prepared using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) following the instructions of the manufacturer. The primers and protocols for *rbc*L amplification and automated sequencing used in this study are given in Lin *et al.* (2001a). New sequence data and those first generated by Lin *et al.* (2001a) were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. Phylogenetic analyses were performed using the maximum parsimony algorithm 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. A set of sequences from 22 representative taxa belonging to the two tribes in the subfamily Nitophylloideae was selected for analysis, with two species of *Phycodrys* Kützing, tribe Phycodryeae, subfamily Phycodryoideae, serving as the outgroup (Table 1). Parsimony heuristic searches and calculation of bootstrap proportion values were conducted as described in Lin *et al.* (2001a): initial searches consisted of 100 random sequence additions, MULPARS (but holding five trees at each step), STEEPEST DESCENT and nearest neighbour interchange (NNI) branch-swapping trees leading to the most parsimonious solution (Maddison 1991). Trees found in these initial searches were then used as starting points for further searches with MULPARS, STEEPEST DESCENT and the tree bisection–reconnection (TBR) algorithm until final swapping was complete. The searches were done on each data set under the criterion of equal weights for all substitutions. Character weighting, unequal weighting of nucleotide characters and different transition–transversion ratios did not significantly alter tree topology. Consistency (CI) and retention (RI) indices (Farris 1989; Kluge & Farris 1989) were calculated excluding uninformative characters. Support for nodes of parsimony trees was assessed by calculating 500 bootstrap resamplings of the heuristic searches based on random stepwise additions, MULPARS and TBR. Decay indices (Bremer 1988) representing the number of steps less parsimonious than minimal at which branches were no longer resolved were determined based on strict consensus analysis of cladograms found by relaxing parsimony sequentially one step at a time, up to five steps.

OBSERVATIONS

Martensia lewisiae Lin, Hommersand & Fredericq, *sp. nov.*

Figs 1–27, 53–55

Thalli formantes tegetes laminarum prostratarum decumbentium lobatarum; laminae sine stipite hapteroque, omnes usque ad 5 cm longae 3–9 mm lataeque, teges plus quam 15 cm latae, vel plures specimina coalescentia, affixae per tenacula discoidea infra laminas prostratas et secus margines membranaceas; laminae membranaceae monostromaticae omnino, reticulis absentibus aut praesentibus in aliquot lobis thallorum maturorum; fila conjungentia evoluta secundatim lamellas oppositas; gametophyta dioecia; sori masculini parvi in maculis irregularibus circularibus thalli dispersi utrinque strati centralis laminae fertilium; procarpia cystocarpiaque in reticulo formata secus marginem laminae vel margines lamellarum longitudinalium; procarpia diagonaliter opposita in utroque pagina laminarum fertilium vel lamellarum, constantia ex cellula obtecta unica, etiam cellula sustinenti ferenti lateralem turmam sterile unicellulatum et filum carpogoniale leviter curvatum 4-cellulatum; fecundatio non visa; cellula basalis et cellulae gonimoblasti uninucleatae, sine conjunctionibus cellularum; carposporangia terminalia in filis subdichotomiramosis gonimoblasti; sori tetrasporangiorum parvi, circulares, plerumque dispersi in partibus membranaceis laminae, raro in reticulo.

Thalli forming extensive tangled mats composed of prostrate and overlapping decumbent lobed blades lacking stipe and holdfast; individual blades up to 5 cm long by 3–9 mm wide, mats more than 15 cm wide, or several specimens coalescent to cover an entire coral head, attached to the substratum by discoid haptera beneath the prostrate blades and along the margins; blades membranous and monostromatic throughout with network absent or present in some lobes of mature thalli; uppermost cells becoming vacuolate, uniting by secondary pit connections and dividing to reform the margin; cross-connecting strands developing unidirectionally and linking to adjacent lamellae on opposite side; gametophytes dioecious; spermatangial sori minute in irregular circular patches scattered over fertile membranous blades on both sides of central layer; procarps and cystocarps formed in network along the margin of the blade or longitudinal lamellae; procarps borne diagonally opposite each other from fertile central cell on both sides of fertile blade or lamellae.

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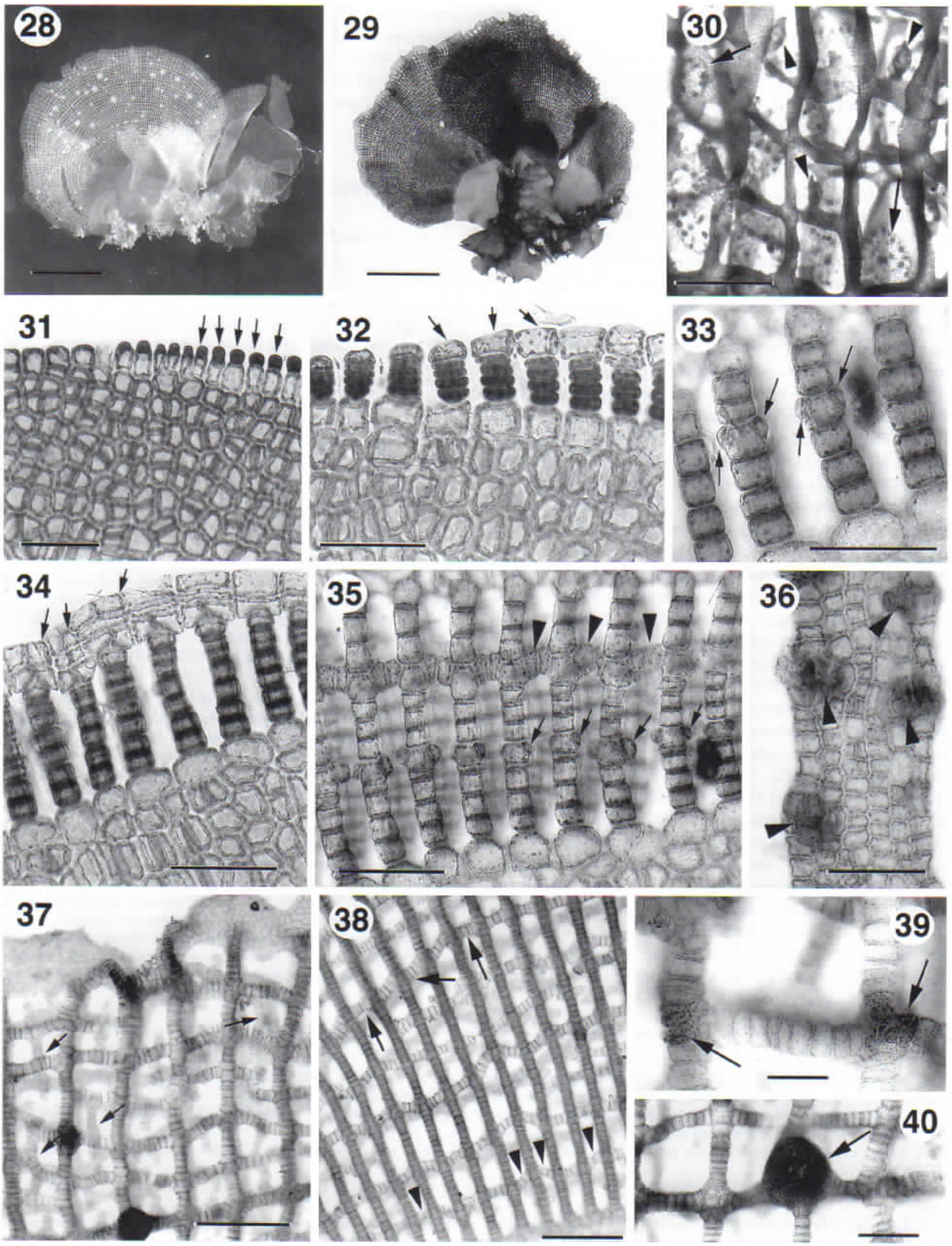
Fig. 18. Early postfertilization stage showing the supporting cell (sc) with an enlarged, darkly staining nucleus, the auxiliary cell (aux) and the gonimoblast initial (gi). Surrounding multinucleate cells are initiating the pericarp. Bar = 50 μm.
Fig. 19. Developing gonimoblasts showing the enlarged pit connection (arrow) between the supporting cell (sc) and the auxiliary cell (aux) and inner gonimoblast cells containing enlarged nuclei (arrowheads). Bar = 50 μm.
Fig. 20. Young cystocarp showing inner gonimoblast cells with enlarged nuclei and differentiating terminal carposporangia (arrowheads). Bar = 100 μm.
Fig. 21. Cross-section of a cystocarp showing that the inner gonimoblast cells (arrows) have not united to form a single fusion cell. Bar = 250 μm.
Fig. 22. Close-up of carposporophyte showing the basal supporting cell (bc), the enlarged nuclei of the inner gonimoblast cells (arrows), the uninucleate outer gonimoblast filaments and the terminal carposporangia (arrowheads). Bar = 100 μm.
Fig. 23. Surface view of tetrasporangial sori in the blade portion of the thallus. Bar = 300 μm.
Fig. 24. Multinucleate tetrasporocyte. Bar = 50 μm.
Fig. 25. Stage in the development of a multinucleate tetrasporocyte. Bar = 50 μm.
Fig. 26. Mature tetrasporocyte containing a single remaining nucleus (arrow). Bar = 100 μm.
Fig. 27. Mature tetrasporangium with tetrahedrally arranged tetraspores (arrow). Bar = 100 μm.



Figs 28–40. *Martensia formosana*. Habit and vegetative and reproductive morphology.
Fig. 28. Holotype; cystocarpic plant. Bar = 1 cm.
Fig. 29. Isotype; tetrasporangial plant. Bar = 1 cm.
Fig. 30. Tetrasporangial sori borne on longitudinal lamellae (arrows) and on secondary lamellae (small blades, arrowheads) on cross-linking strands inside the network. Bar = 1 mm.

consisting of a cover cell and a supporting cell bearing a one-celled sterile lateral and a slightly curved, four-celled carpogonial branch; fertilization not seen; basal supporting cell and gonimoblast cells uninucleate with cell fusions absent; carposporangia terminal on subdichotomously branched gonimoblast filaments; tetrasporangial sori small, circular, mostly scattered over membranous parts of blade, rarely on network; tetrasporangial initial multinucleate, transformed from a cell in monostromatic blade or lamella.

HOLOTYPE: NMMA, 3-28-2002-BB-ML-Holo (Fig. 1).

ISOTYPES: LAF, 3-28-2002-BB-ML-Iso-1~3; NMMA 3-28-2002-BB-ML-Iso-4~7.

TYPE LOCALITY: Banana Bay, Kenting National Park, southern Taiwan (21°55.50′N, 120°49.44′E).

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

HABITAT AND SEASONALITY: Collections were made in October 2001 and January, March and October 2002. Absence of perennial stipes indicates that this taxon may be an annual. Plants grew seasonally from autumn to early spring, mainly at 1–3 m depth, where they were abundant on coral heads or were epiphytic on the red alga *Gelidiopsis repens* (Kützing) Weber-van Bosse and some other red algae at the same depth.

ETYMOLOGY: 'Lewisiae' is in honour of Dr Jane E. Lewis, prominent phycologist in Taiwan, who introduced the first author to the beautiful world of the marine algae of Taiwan.

SPECIMENS EXAMINED: Banana Bay (21°55.50′N, 120°49.93′E), 1–3 m depth, coll. *S.-M. Lin*, tetrasporic, 29 October 2001; tetrasporic, 8 January 2002; spermatangial, cystocarpic, tetrasporic, 14 March 2002; cystocarpic (holotype), tetrasporic (isotypes), 28 March 2002; cystocarpic, tetrasporic, 2 October 2002. White Sand Bay (21°56.11′N, 120°42.96′E), 1–3 m depth, coll. *Jack Huang*, tetrasporic, 13 February 2001.

HABIT AND VEGETATIVE MORPHOLOGY: The thalli are composed of prostrate and overlapping decumbent, lobed blades (Figs 1, 2) and form mats 2–4 cm high and up to more than 15 cm in width, or several specimens coalesce to cover an entire coral head. The blades are bright red with pink and blue iridescence. Decumbent blades are subdichotomously to irregularly lobed, and the free ends are unevenly 2–9 mm wide by 5–20 mm long, so that most plants have a highly irregular outline (Figs 1, 2). Margins are entire (Fig. 3) or have microscopic teeth. Blades are membranous and monostromatic throughout except for the reproductive portions and measure 15–25 μm thick (Fig. 6).

Growth is diffuse, caused by the meristematic activity of multinucleate marginal and intercalary cells (Fig. 3). Networks were not seen in young thalli, and were only weakly developed when thalli had matured. Only a single network band is formed, 1–3 mm high and 2–8 mm wide, and it has a membranous margin (Figs 4, 5). A network is initiated from a row of transformed marginal cells that are vacuolate at the base and densely filled with cytoplasm at the tip (Fig. 3). Each cell cuts off an apical initial that divides transversely, followed by transverse division and acropetal maturation of the intercalary cells (Fig. 4). The topmost cells become vacuolate, unite laterally by secondary pit connections and divide irregularly to form a new membranous margin (Fig. 5). Cells within the intercalary filaments that were initially only one cell wide divide longitudinally and transversely perpendicular to the plane of the blade and become longitudinal lamellae up to 16–22 cells wide (200–330 μm, Figs 7, 8). Cross-connecting strands (Fig. 7, arrows) are initiated from the edges and centre of the longitudinal lamellae when they become six to eight cells wide, grow unilaterally and attach to adjacent lamellae to form the network (Fig. 5). The cross-connecting strands consist initially of filaments and broaden into lamellae later. Second-order longitudinal lamellae may originate from the middle of the cross-connecting lamellae inside the net (Fig. 8, arrows) and are usually weakly developed. A succession of parallel meshes is formed within the thallus margin, and new meshes are added mainly toward the base of the growing network (Fig. 10). A holdfast or stipe is absent and the blades are anchored directly to the substratum by discoid haptera borne on the undersurface of prostrate blades or on the blade margins (Fig. 9, arrows). There are numerous, discoid or ellipsoid plastids arranged parietally in each surface cell, and secondary pit connections are abundant between adjacent multinucleate cells.

REPRODUCTIVE MORPHOLOGY: Gametophytes are dioecious and similar to the tetrasporophytes (isomorphic). Spermatangial sori are formed on both sides of fertile blades and are minute and irregularly discoid in shape, 100–200 μm in diameter (Fig. 10, arrows; Fig. 11). Surface cells divide anticlinally five to six times to form successively smaller cells, which mature into spermatangial parent cells on both sides of the fertile area (Fig. 12). Each parent cell (Fig. 13, arrowheads) divides obliquely to cut off one to two spermatangia (Fig. 13, arrows) that become 6–7.5 μm long × 2.5–3.5 μm wide. Pores (Fig. 10, arrowheads) are left after spermatangial release.

Procarps are abundant near the margins of the membranous

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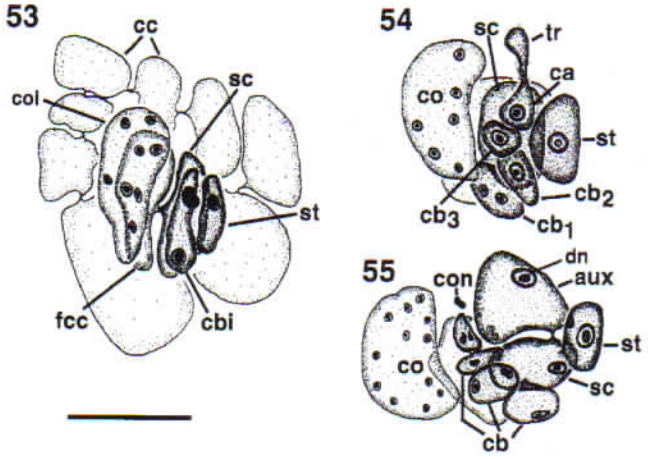
Figs 31–38. Network formation.
Fig. 31. Early stage in network formation showing the initials (arrows) of cell rows at the thallus margin. Bar = 100 μm.
Fig. 32. Elongation of the cell rows by intercalary cell divisions (darkly staining cells) and the initiation of a membranous marginal meristem (arrows) consisting of enlarged vacuolate cells. Bar = 100 μm.
Fig. 33. Bilateral initiation (arrows) of the cross-connecting strands between longitudinal lamellae. Bar = 100 μm.
Fig. 34. Growth of membranous margin by intercalary cell divisions (arrows) which become linked laterally by secondary pit connections. Bar = 100 μm.
Fig. 35. Developing network showing the oldest cross-connecting strands (arrowheads) to the outside and second-layer initials of cross-connecting strands (arrows) to the inside of the network. Bar = 100 μm.
Fig. 36. Surface view of a longitudinal lamella showing attachment points (arrowheads) of cross-connecting strands. Bar = 150 μm.
Fig. 37. Distal portion of a network showing primary lamellae, cross-connecting strands and secondary longitudinal filaments (arrows) issuing from the cross-connecting strands. Bar = 100 μm.
Fig. 38. Proximal portion of a network showing longitudinal lamellae linked by older cross-connecting strands (arrows) and newly formed cross-connecting strands (arrowheads) toward the base. Bar = 100 μm.
Fig. 39. Procarps (arrows) on intersecting cross-connecting strands and longitudinal lamellae. Bar = 100 μm.
Fig. 40. Young cystocarp on intersection of lamellae. Bar = 200 μm.

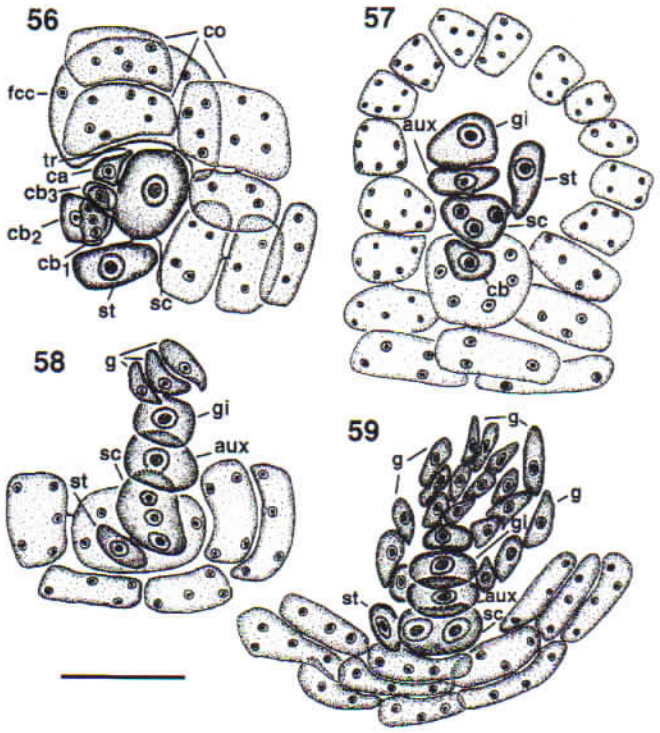


Figs 53–55. *Martensia lewisiae.* Procarp structure. Bar = 25 μm.

Fig. 53. Young procarp showing central cells (cc) including the fertile central cell (fcc) below the cover cell initial (coi) and the supporting cell (sc) bearing a one-celled sterile lateral (st) and a lateral carpogonial branch initial (cbi).

Fig. 54. Mature procarp consisting of a multinucleate one-celled cover cell (co), a supporting cell (sc) bearing a one-celled sterile lateral (st) and a four-celled carpogonial branch (cb1, cb2, cb3) with carpogonium (ca) and terminal trichogyne (tr).

Fig. 55. Postfertilization stage showing a supporting cell (sc), lateral cover cell and one-celled sterile lateral (st), a four-celled carpogonial branch (cb), an unfused connecting cell (con) and an auxiliary cell (aux) with diploid nucleus (dn).



Figs 56–59. *Martensia formosana.* Procarp and postfertilization stages. Bar = 25 μm.

Fig. 56. Prefertilization stage showing fertile central cell (fcc) that has cut off a three-celled group of cover cells (co) and a supporting cell (sc) bearing a one-celled sterile lateral (st) and a four-celled carpogonial branch (cb1, cb2, cb3) with carpogonium (ca) and trichogyne (tr).

Fig. 57. Early postfertilization stage showing the supporting cell (sc), the remnants of the carpogonial branch (cb), a one-celled laterobasal sterile cell (st) and an auxiliary cell (aux) that has cut off a gonimoblast initial (gi).

Fig. 58. Later stage showing a supporting cell (sc) bearing a sterile lateral (st) and auxiliary cell (aux) and gonimoblast initial (gi) that has cut off gonimoblast cells (g) by oblique divisions.

Fig. 59. Later stage showing a supporting cell (sc) bearing a sterile lateral (st) and auxiliary cell (aux) and gonimoblast initial (gi) bearing terminal and lateral gonimoblast filaments (g).

the carpogonium cuts off two minute connecting cells, and the supporting cell cuts off a large auxiliary cell. Figure 55 shows a stage in which the diploid nucleus has already entered the auxiliary cell. The carpogonial branch degenerates and the auxiliary cell divides, cutting off a terminal gonimoblast initial (Fig. 18), which grows distally and branches laterally to produce a tightly packed cluster of gonimoblast filaments (Fig. 19). As gonimoblast development progresses, the primary pit connections between the gonimoblast cells and the one linking the auxiliary cell to the supporting cell broaden (Fig. 19, arrow), and the gonimoblast nuclei enlarge (Fig. 19, arrowheads). Gonimoblast cells do not fuse, and a large fusion cell is absent (Figs 20, 21). As a result, the uninucleate gonimoblasts remain intact (Fig. 21, arrows) and connected via a uninucleate auxiliary cell to a persistent, uninucleate basal cell that corresponds to the original supporting cell (Fig. 22). A mature cystocarp is hemispherical and measures 750–870 μm in diameter, and is covered by a three- to five-cell layered pericarp with the ostiole off centre. Pyriform carposporangia measuring 40–60 μm by 100–130 μm are formed terminally (Figs 20, 22, arrowheads).

Tetrasporangial sori are minute, irregularly circular in shape (Fig. 23), 300–625 μm by 300–400 μm in diameter, solitary or slightly aggregated and scattered over both sides of the fertile blades. Some sori are formed inside the network as the network develops. Tetrasporocytes are transformed from multinucleate cells in the monostromatic portion of the blade (Fig. 24); they enlarge and become darkly staining (Fig. 25). Ultimately, all the nuclei but one degenerate (Fig. 26, arrow). Mature tetrasporangia are tetrahedrally divided and 120–135 μm in diameter (Fig. 27, arrow).

Martensia formosana Lin, Hommersand & Fredericq, *sp. nov.*

Figs 28–52, 56–59

Thalli flabelliformes ex 1–3 laminis flabellatis constantes ad substratum per discoideum hapteron affixis; laminae 3–4 cm longae, 3–5 cm latae, reticulo conspicuo unifasciato. Reticulum constans ex lamellis primariis longitudinalibus concatenatisque quae secundarias lamellas concatenatas longitudinalesque includunt; connatae synapsibus secundis et dividentes reformandae marginem; fila conjugentia bilateraliter evoluta mediconcatenata inter lamellas longitudinales; gametophyta dioecia; sori masculini in reticulo portati; procarpia in utroque pagina reticuli portata ad juncturas lamellarum longitudinalium concatenatarumque constantia ex turma cellularum obtectarum, laterali-basali turma sterili et curvato laterali-basali ramo carpogoniali; cystocarpia ad juncturam lamellarum longitudinalium concatenatarumque portata; cystocarpia juvenia constantia ex cellula basali multinucleata et cellula auxiliari uninucleata et cellulis uninucleatis filorum ramosorum gonimoblasti pericarpio centraliostiolato exsuperatis; cystocarpia matura non visa; sori tetrasporangiorum in lamellis reticuli non statim in lamina portati; initium tetrasporangii multinucleatum, transformatum e cellula superficiali.

Thalli fan-shaped, consisting of one to three flabellate blades at-

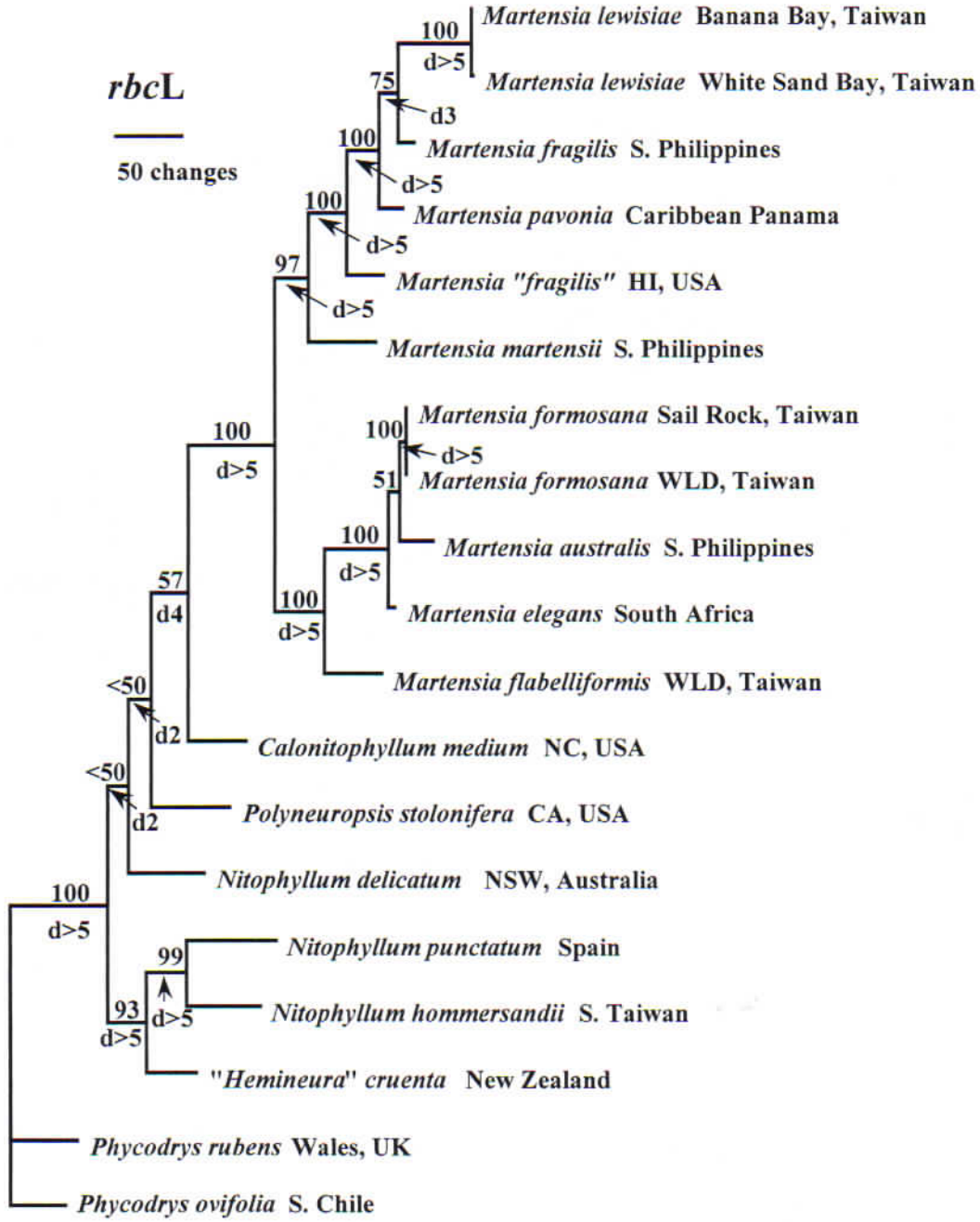


Fig. 60. One of two equally most parsimonious trees from *rbc*L sequence analysis showing nested positions of *Martensia lewisiae* and *M. formosana* within the Martensieae. Tree length = 1102, CI = 0.6089, RI = 0.7063, 326 informative characters out of 1407 included sites. Bootstrap proportion values (1000 replicates, > 50 %) are shown above nodes; decay indices are shown below nodes. Branch lengths are proportional to the amount of sequence change.

tached to the substratum by one to few inconspicuous, discoid holdfasts; blades 3–4 cm in length by 3–5 cm in width with a conspicuous network consisting of a single, continuous band. Network composed of primary longitudinal and cross-connecting lamellae enclosing secondary cross-connecting and longitudinal lamellae; cross-connecting strands developing bilaterally and linking midway between longitudinal lamellae; gametophytes dioecious; spermatangial sori borne on network; procarps borne on both sides of network at the junctions of longitudinal and cross-connecting strands and consisting of a group of cover cells, a laterobasal sterile cell and a curved, laterobasal carpogonial branch; cystocarps developing at the juncture of longitudinal and cross-connecting lamellae; young cystocarps consisting of a multinucleate basal cell, a uninucleate auxiliary cell and uninucleate cells of branched gonimoblast filaments surmounted by a pericarp with a central ostiole; mature cystocarps not seen; tetrasporangial sori borne on primary and secondary lamellae in network and not directly on blade; tetrasporangial initial multinucleate, transformed from a lamellar cell.

HOLOTYPE: NMMA, 10-02-2001-SR-Holo (Fig. 28).

TYPE LOCALITY: Sail Rock, Kenting National Park, southern Taiwan (21°55.97′N, 120°49.44′E).

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

HABITAT AND SEASONALITY: Plants were rare in October, November and December 2001; depth 1–5 m on coral heads.

ETYMOLOGY: 'Formosana' refers to Taiwan, where this new red alga was found.

SPECIMENS EXAMINED: Sail Rock (21°55.97′N, 120°49.44′E), 1–2 m, coll. *S.-M. Lin*, cystocarpic (holotype), 2 October 2001; tetrasporic (paratype), 29 November 2001. Wan-Lee-Dong (21°59.77′N, 120°42.29′E), 5 m, coll. *S.-M. Lin*, tetrasporic, spermatangial (paratype), 20 December 2001.

HABIT AND VEGETATIVE MORPHOLOGY: The thalli are fan-shaped and consist of one to three flabellate blades, 3–4 cm high and 3–5 cm wide; they are attached to rocks by several small discoid holdfasts at the base, and have a network consisting of a single continuous band and membranous margin that extends over 60–80% of the entire blade (Figs 28, 29). Blades are bright red to rose-red in colour; the membranous parts are one- to two-cell layered and 95–105 μm thick. The margins are toothed or entire.

Growth is diffuse, caused by meristematic activity of multinucleate marginal and intercalary meristems (Fig. 31). A network is initiated from a row of transformed marginal cells that are vacuolate at the base and densely filled with cytoplasm at the tip (Fig. 31, arrows). Each cell cuts off an apical initial that divides transversely, followed by transverse division and acropetal maturation of the intercalary cells (Figs 31, 32). The uppermost cells become vacuolate and unite by secondary pit connections, and then undergo intercalary cell divisions to reform a membranous margin (Figs 32, 34, arrows). Cross-connecting strands are initiated bidirectionally from the edges and inside the margins of the primary longitudinal lamellae where the lamellae are four to six cells wide (Fig. 33, arrows), and meet in the space between the lamellae where they are united by secondary pit connections (Fig. 35, arrowheads). Expansion of the network is by intercalary cell divisions of primary longitudinal lamellae and the continued formation of cross-connecting strands (Figs 35–38). A surface view of a longitudinal lamella shows attachment points (Fig. 36, arrowheads) of cross-connecting strands. New cross-linking strands originate at the base of the network in association with the elongation of primary longitudinal lamellae (Fig. 35, arrows). Primary longitudinal lamellae and cross-connecting lamellae are sheet-like, 8–20 cells (625–875 μm) wide. Secondary longitudinal lamellae (Fig. 37, arrows) are initiated from the middle of the cross-linking lamellae filling in the space created by the expansion of the primary network. Older lamellae (Fig. 38, arrows) undergo additional intercalary cell divisions and issue secondary cross-linking filaments as the network expands (Figs 37, 38, arrowheads).

REPRODUCTIVE MORPHOLOGY: Gametophytes are dioecious and isomorphic, similar to the tetrasporophytes (isomorphic). Procarps (Fig. 39, arrows) and cystocarps are formed in the intersections of lamellae and cross-connecting strands (Figs 39, 40). A fertile central cell cuts off two pericentral cells, one of which functions as the cover cell initial and the other as the supporting cell. Procarps consist of a group of cover cells and a supporting cell bearing a laterobasal sterile cell and a slightly curved laterobasal four-celled carpogonial branch (Figs 41–42, 56). After presumed diploidization, the auxiliary cell cuts off a terminal gonimoblast initial (Fig. 57) that divides obliquely (Figs 43, 58). Secondary gonimoblast initials arise laterally from the auxiliary cell and the gonimoblasts (Fig. 44, arrows) and branch subdichotomously. Although the primary pit connections broaden, the gonimoblast cells remain distinct and do not fuse. The gonimoblasts are attached through the auxiliary cell to a multinucleate basal cell that corresponds to the original supporting cell (Figs 44, 59). Mature cystocarps were not seen in our material.

Spermatangial sori are formed on the primary longitudinal lamellae of the fertile blades and are 100–700 μm long and 60–270 μm wide (Fig. 45). Surface cells on both sides of fertile longitudinal lamellae divide five to seven times to form successively smaller cells, which mature into spermatangial parent cells (Fig. 46). Each spermatangial parent cell cuts off one to two spermatangia, 5–6 μm long by 2–3 μm wide (Fig. 47, arrows).

Tetrasporangial sori are formed on primary longitudinal lamellae (Fig. 30, arrows) and on bladelets (Fig. 30, arrowheads) formed from secondary longitudinal lamellae that have arisen from cross-linking strands. Tetrasporangial sori are rounded to ovoid in shape (Fig. 48), 300–800 μm by 400–1125 μm in diameter and solitary or aggregated. Tetrasporocytes are transformed from multinucleate lamellar cells (Fig. 49, arrowhead) that have enlarged and become connected to neighbouring cells by numerous secondary pit connections (Fig. 49, arrows). In the course of their development, the tetrasporocytes are covered by cells cut off from neighbouring cells (Fig. 50, arrows). Each tetrasporocyte becomes uninucleate through nuclear degeneration, leaving behind a single functional nucleus (Fig. 51, arrow). Mature tetrasporangia are tetrahedrally divided and measure 120–130 μm in diameter (Fig. 52).

Molecular analyses

The *rbc*L sequences of *M. lewisii* sp. nov. and *M. formosana* sp. nov. from Kenting National Park, southern Taiwan (type locality), were newly generated. A set of 15 additional representative taxa belonging to the two tribes, Martensieae and Nitophylleae, in the subfamily Nitophylloideae were selected for the analysis (Table 1) together with two species of *Phycodrys* in the subfamily Phycodryoideae, which served as the outgroup (Fig. 60). Selection of all the taxa in the Phycodryoideae or any of the several other equally basal taxa in the Phycodryoideae did not change the topology of the trees. The final *rbc*L data matrix was restricted to 1407 sites. Parsimony analysis revealed two most parsimonious trees with tree length of 1102 steps, CI = 0.6089 and RI = 0.7036; there were 393 informative characters out of 1407 included sites (28%). Bootstrap proportion values (1000 replicates, > 50%) and decay indices derived from maximum parsimony analysis are shown on the nodes. Branch lengths are proportional to the amount of sequence change.

Interspecific *rbc*L sequence divergence among species of the Martensieae clade varied from 1.3% to 13.6%. *Martensia lewisii* is sister to *M. fragilis* from the southern Philippines (75% bootstrap support) with 5.5% sequence divergence (75 characters), whereas *M. formosana* is closely related to *M. australis*, also from the southern Philippines, with 2.2% sequence divergence (31 characters) and to *M. elegans* from South Africa with 1.3% sequence divergence (18 characters).

DISCUSSION

Our understanding of the comparative morphology of *Martensia* species is largely due to the work of Svedelius (1908), who investigated the process of network formation and reproductive development in male, female and tetrasporangial thalli in several species of *Martensia*. Most notably, he described an unusual pattern of tetrasporocyte formation in *M. fragilis* and again in *Nitophyllum punctatum* (Stackhouse) Greville (Svedelius 1914), in which the tetrasporocyte initial is an ordinary multinucleate cell in the monostromatic membrane of the blade or lamella that is multiply pit-connected to neighbouring cells. Differentiation involves cell enlargement, an increase in cytoplasmic content and the disintegration of all but one nucleus, which according to Svedelius, undergoes meiosis to produce four tetraspores. This pattern of tetrasporangial formation was confirmed in *M. lewisiae* and *M. formosana* and is probably diagnostic for *Martensia* and some species of *Nitophyllum*.

Criteria used for separating species consist of combinations of the following characters: number and shape of the blades, whether rounded, fan-shaped, lobed or deeply lacerate; network construction, whether formed in a single band or multiple bands; blade margins, whether toothed or entire; reproductive structures, whether restricted to the networks or blades, or present on both networks and blades. The orientation of the cross-connecting strands may provide a useful taxonomic character. They have been described as being initiated unidirectionally in *M. pavonia* by Børgesen (1919) and are unidirectional in *M. lewisiae*. Their formation is bidirectional in *M. fragilis* (Svedelius 1908) and *M. formosana*. Variations may occur in this feature and its taxonomic significance is unclear at this time.

Selecting *Phycodrys* (one of several basal taxa in the Phycodryeae) as the outgroup, the tribe Martensieae is shown to be monophyletic with strong (100%) bootstrap support and to consist of sister clades receiving 100% and 97% bootstrap support (Fig. 60). If the two clades identified in the molecular tree were recognized at the generic level, one would correspond to *Martensia sensu* Hering, type species *M. elegans* (Hering 1841) from Point Natal (= The Bluff, Durban), Natal, South Africa, and the other would be assigned to *Mesotrema* J. Agardh (Agardh 1854), type species *Mesotrema pavonia* J. Agardh from St Croix in the Western Atlantic Ocean. Should future studies support the recognition of two genera or subgenera, they would probably be separated morphologically on the basis of the position of the procarp and cystocarp in the network and on features of procarp and cystocarp development.

The clade containing *Martensia formosana* includes the type species *M. elegans* from South Africa, *M. australis* from the Philippines and *M. flabelliformis* from Taiwan. *Martensia formosana* stands closest to *M. elegans* and *M. australis* based on sequence divergences of 1.3% and 2.2%, respectively. It differs morphologically from *M. elegans* in its habit and in the location of the tetrasporangial sori. Whereas the basal membranous part of the thallus of *M. elegans* is foliose, attached by haptera and with individual blades sometimes stipitate (Hering 1844, pl. 7, figs 1–3; Svedelius 1908, pl. 1, fig. 12), the thallus of *M. formosana* is sessile, attached by one to few small discoid holdfasts. The tetrasporangia form in small sori, either directly on the blades or in the longitudinal lamellae in *M. elegans* (Hooker 1844, pl. 697), whereas they are absent from the blade and either occur in the longitudinal lamellae or, more often, occur on bladelets corresponding to secondary longitudinal lamellae that are borne on the cross-connecting strands in *M. formosana*. *Martensia formosana* is distinguishable from *M. australis* mainly by its size, with the former reaching 4–5 cm in height, whereas the latter can be up to 20 cm tall.

The clade containing *M. lewisiae* includes *M. pavonia* from the Caribbean Sea, two species assigned to *M. fragilis*, and *M. martensii* (formerly *Opephyllum martensii*) from the Philippines. *Martensia lewisiae* can be separated from all other species by its tendency to form extensive mats from a creeping system of prostrate blades attached by basal and marginal haptera, and by a weakly developed network that seems to form only in mature reproductive thalli, especially on female gametophytes. It is most closely related to *M. fragilis* from the southern Philippines. Further studies will show whether or not the decision by Millar (1990) to place *M. denticulata* and *M. pavonia* in synonymy under *M. fragilis* is justified. There may well be a pantropical species with multiple network bands that would be referable to *M. fragilis*. On the other hand, our molecular observations, some of which are presented in Fig. 60, suggest that separate species may have gone unrecognized. A solution to the species problems will require an investigation of critical stages of vegetative and reproductive development in topotype material that has been compared in each case with the type.

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