

# CHARACTERIZATION OF *MARTENSIA* (DELESSERIACEAE, RHODOPHYTA) BASED ON A MORPHOLOGICAL AND MOLECULAR STUDY OF THE TYPE SPECIES, *M. ELEGANS*, AND *M. NATALENSIS* SP. NOV. FROM SOUTH AFRICA<sup>1</sup>

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An examination of a series of collections from the coast of Natal, South Africa, has revealed the presence of two species of *Martensia* C. Hering nom. cons: *M. elegans* C. Hering 1841, the type species, and an undescribed species, *M. natalensis* sp. nov. The two are similar in gross morphology, with both having the network arranged in a single band, and with reproductive thalli of *M. elegans* usually larger and more robust than those of *M. natalensis*. Molecular studies based on *rbcl* sequence analyses place the two in separate, strongly supported clades. The first assemblage occurs primarily in the Indo-West Pacific Ocean, and the second is widely distributed in tropical and warm-temperate waters. Criteria that have been used in the past for separating the two, namely, the number and shape of the blades, the presence of a single- versus a multiple-banded network, and blade margins entire or toothed, were determined to be unreliable. Although the examination of additional species is required, the morphology and position of procarps and cystocarps, whether at or near the corners of the longitudinal lamellae and the cross-connecting strands or along the lobed, membranous edges of the longitudinal lamellae or on the thallus margins, may prove to be diagnostic at the subgenus level. We recognize subg. *Martensia*, including the type of *Martensia*: *M. elegans* and subg. *Mesotrema* (J. Agardh) De Toni based on *Martensia pavonia* (J. Agardh) J. Agardh.

**Key index words:** Delesseriaceae; *Martensia*; *Martensia elegans*; *Martensia natalensis* sp. nov.; Rhodophyta; subgenus *Martensia*; subgenus *Mesotrema*; systematics

**Abbreviations:** *M.*, *Martensia*; *rbcl*, large subunit of the RUBISCO gene; subg., subgenus

The genus *Martensia* was established with a brief diagnosis by Hering (1841) based on plants collected by Dr. Ferdinand Krauss on rocks at Port Natal (present-day Durban) in South Africa. Hering (1844), published posthumously by Krauss, contains a more detailed description and illustrations of *M. elegans*, particularly of cystocarpic material. *Hemitrema* R. Brown ex Endlicher (1843) [type species: *H. kraussii*] was proposed based on material collected by Krauss. W. J. Hooker (1844) observed that the tetrasporangia were borne in both the longitudinal lamellae of the network and also in the proximal membrane in the Krauss material and concluded that the tetrasporangia were comparable to those found in *Nitophyllum*. Harvey (1849), like Hooker, had originally thought that *M. elegans* resembled *Nitophyllum*; however, upon observing that female plants possessed ovate, osteolate cystocarps bearing pyriform carposporangia, he concluded that *Martensia* belonged in the Rhodomeleae near the genus *Claudea*.

J. Agardh (1854) recognized two genera, *Martensia* C. Hering and a new genus *Mesotrema* (type species: *Mesotrema pavonia* J. Agardh) based on a collection from St. Croix in the Virgin Islands. The two genera were separated on the grounds that the network consisted of a single zone in *Martensia* and several (two or more) zones separated by membranous regions in *Mesotrema*. J. Agardh (1863) revised his concept of *Martensia*, which he placed in his Ordo XVI. Rhodomeleae (=Rhodomeleaceae), Tribus 2. Pollexfenieae. He recognized a single genus, *Martensia* C. Hering, containing two

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divisions: (1) *Hemitrema*, in which the network forms a single band, and (2) *Mesotrema*, in which the network is multiple banded. Finally, J. Agardh (1885) established Ordo Martensiaceae to contain *Martensia*, which he placed next to Ordo Rhodomeleae based on the structure of the cystocarp. The classification of *Martensia* was clarified by Schmitz and Hauptfleisch (1897), who placed the genus in the Delesseriaceae.

De Toni (1936) argued that *Martensia* C. Hering (1841) is invalidated by *Martensia* Giseke 1792 and that *Hemitrema kraussii* R. Brown ex Endlicher 1843, being based on *M. elegans* C. Hering, is also invalid and proposed *Capraella* as a substitute name for *Martensia* [Type species: *C. elegans* (Hering) De Toni]. Later, Papenfuss (1942) pointed out that De Toni (1936) had overlooked *Mesotrema* J. Agardh 1854, type species *Mesotrema pavonia* J. Agardh 1854, and he transferred all the recorded species to *Mesotrema*. Recognizing the confusion caused by all the name changes, Silva (1950) proposed *Martensia* C. Hering for conservation, a proposal that has been adopted.

The status of *Martensia* C. Hering 1841 is complicated by the fact that two different species, both from the type locality, Kwazulu-Natal, go under the same name at present. One of these corresponds to *M. elegans* C. Hering, the type species of *Martensia*. The other, *M. natalensis* sp. nov., could be related to *M. fragilis* Harvey (1854) from Sri Lanka. Svedelius (1908) examined these two species of *Martensia* in the most detailed investigation of network and reproductive development carried out so far. Our study reexamines the features of vegetative and reproductive morphology of *Martensia* in comparison with the observations of Svedelius and our previous studies of *Martensia formosana* Lin, Hommersand et Fredericq and *M. lewisiae* S.-M. Lin, Hommersand et Fredericq from Taiwan (Lin et al. 2004a). Phylogenetic and taxonomic conclusions are drawn from morphological observations and from *rbcl* sequence analyses of a range of *Martensia* species.

#### MATERIALS AND METHODS

Specimens were collected intertidally from pools or subtidally by snorkeling or SCUBA diving. Samples used in morphological studies were preserved in 5% formalin/seawater or pressed on herbarium sheets. Voucher specimens were deposited in the herbaria of the University of North Carolina at Chapel Hill (NCU); the Herbarium in Ghent, Belgium (GENT); and the National Taiwan Ocean University, Taiwan (NTOU). Hand sections were stained with 1% aniline blue acidified with 1% HCl and mounted in 25%–30% Karo<sup>®</sup> syrup (Englewood Cliffs, NJ, USA) or were treated with Wittmann's aceto-iron-hematoxylin-chloral hydrate (Wittmann 1965) and mounted in 50% Hoyer's mounting medium (Lin et al. 2001a). After 1–2 h, the whole-mount slides were then placed in an alcohol-xylene series, if applicable, and mounted in 50% Piccolyte<sup>®</sup>-xylene solution (Ward's Natural Science Establishment Inc., Rochester, NY,

USA). This procedure clears the material so that it is possible to focus through several layers of cells revealing the internal structure (Hommersand and Fredericq 1997). Photomicrographs were taken on an Olympus BX51 microscope with a Q-imaging digital camera (Burnaby, BC, Canada), and habit views were reproduced with an Epson scanner (Tokyo, Japan). Herbarium abbreviations follow Holmgren et al. (1990).

Specimens dried in silica gel were extracted for DNA analysis. DNA samples were prepared using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the instructions of the manufacturer. DNA-sequencing procedures are as described in Lin et al. (2001b). New sequence data and those available from GenBank were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. Nine *rbcl* sequences were newly generated in this study from collections of *M. elegans* and *M. natalensis* sp. nov. from Natal, South Africa, and compared with those of other *rbcl* sequences of *Martensia* and five other members of the subfamily Nitophylloideae available from GenBank (see Table S1 in the supplementary material). Phylogenetic analyses were performed using parsimony heuristic searches (the maximum parsimony [MP]), and calculation of bootstrap proportion values (BP) were conducted as described in Lin et al. (2001b). MP analysis and bootstrapping methods are available in the computer programs PAUP\* v4.0 (Swofford 2002), with 1,000 bootstrap replicates completed for the MP analysis.

Heuristic maximum-likelihood (ML) searches and bootstrap analyses (1,000 replicates) were run in PHYML (Guindon and Gascuel 2003), using a GTR + I +  $\Gamma$  model with parameters estimated by the program: base frequencies A = 0.324, C = 0.166, G = 0.207, T = 0.301; substitution rates A-C = 1.271, A-G = 4.417, A-T = 1.746, C-G = 0.997, C-T = 13.468, G-T = 1.0; proportion of invariable sites in the alignment is 0.000, and the shape parameter of the gamma distribution among site rate heterogeneity is 0.223. A Bayesian analysis was performed in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a GTR + I +  $\Gamma$  model, which allowed for rate variation among different codon positions. Posterior probabilities were estimated using a Metropolis-coupled Markov chain Monte Carlo approach with sampling according to the Metropolis-Hastings algorithm. The analysis used four chains, one cold and three incrementally heated. Each run consisted of  $2 \times 10^6$  generations and was sampled every 1,000th generation. Burn-in values were set at 500,000 generations.

#### RESULTS

**Morphological observations.** *Martensia elegans* Hering 1841:92 (Figs. 1–5).

**Synonyms:** *Hemitrema kraussii* R. Brown ex Endlicher 1843, *Capraella elegans* (Harvey) J. De Toni f.1936; *Mesotrema elegans* (C. Hering) Papenfuss 1942.

**Lectotype:** A cystocarpic plant at BM in the lower left-hand corner (Fig. 1a) on a paper cutout containing six plants carrying the number N. 272 (lower right, barcode BM 000774894) on a herbarium sheet containing a collection referred to by Hering (1841, p. 92) "as the number prefixed to this species in Dr. Krauss's Fasciculi of Natal Specimens that were forwarded to the Botanical Society of London where they may be consulted on application" (Art. 9.2, Vienna Code).

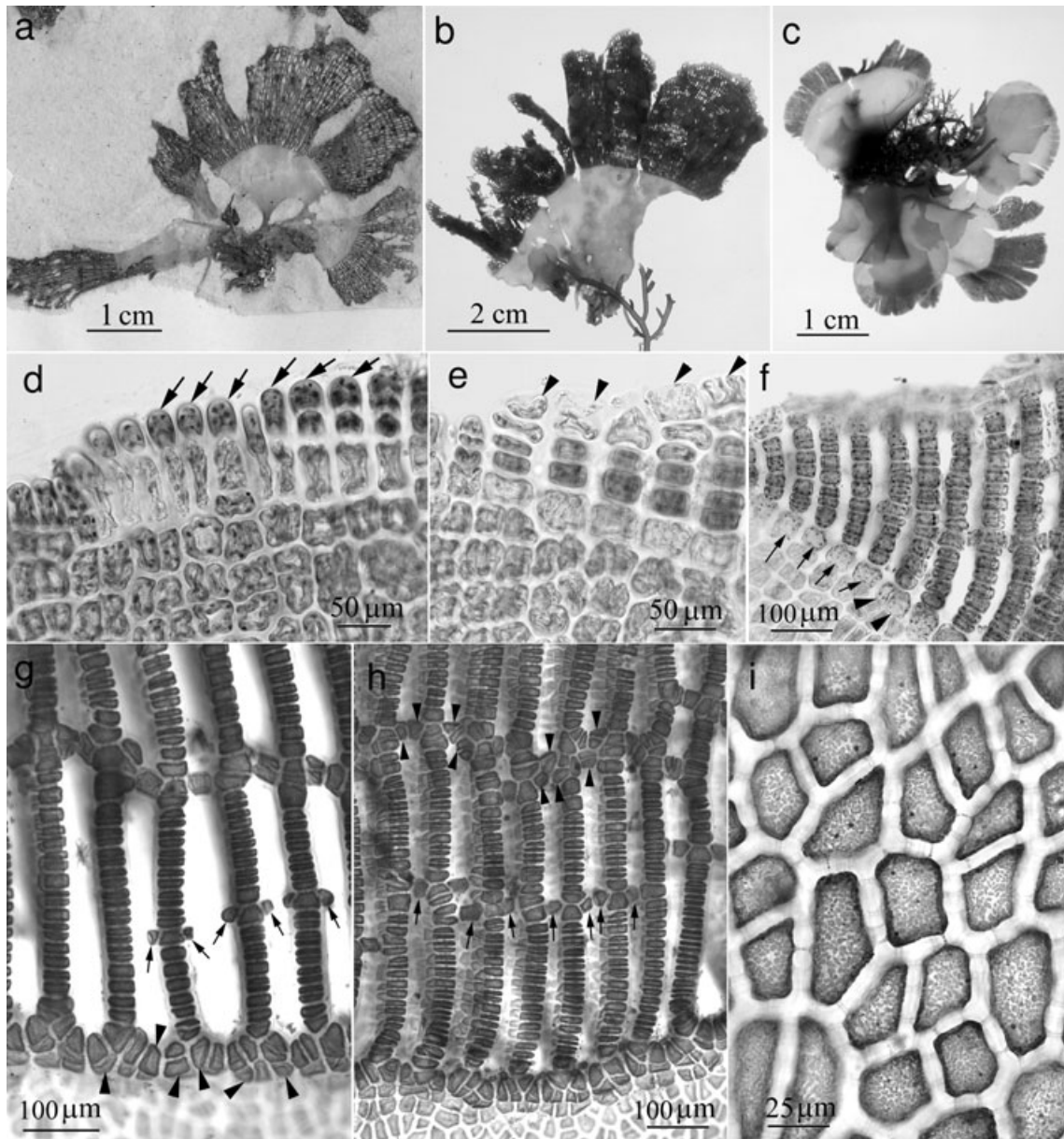


FIG. 1. *Martensia elegans* (South Africa). Habit and vegetative morphology. (a) Lectotype from Port Natal (barcode BM 000774894), a female plant showing flabellate blades with a single-banded network. (b) Female plant from Mzamba, E. Cape. (c) Young thallus from Palm Beach, Natal, with narrow single-banded networks. (d) Early stage in network formation showing initials (arrows) of longitudinal lamellae along the thallus margin. (e) Elongation of cell rows of longitudinal lamellae by intercalary cell divisions and initiation of a membranous marginal meristem (arrowheads) consisting of enlarged vacuolated cells. (f) Early stage of network formation, noting that basal cells (arrows) of longitudinal lamellae undergo longitudinal cell divisions (arrowheads). (g) Further development of network showing bilateral initiation (arrows) of cross-connecting strands between longitudinal lamellae and further cell divisions of basal cells (arrowheads) of longitudinal lamellae. (h) Basal portion of developing network, showing the formation of cross-connecting strands by pit-connections between cells (arrows) derived from longitudinal lamellae on opposite sides. (i) Close-up of surface cells showing discoid plastids and numerous secondary pit-connections.

*Isotypes*: The five other plants on the sheet labeled N. 272 (barcode BM 000774894) that contains the lectotype (Art. 9.10, Vienna Code).

*Historical collections*: Dr. Ferdinand Krauss of Stuttgart distributed many samples mounted on mica of his new species collected on rocks at Port Natal (see Appendix S1 in the supplementary material). Early

distributions went to Hering in Hamburg and to Robert Brown who described *Hemitrema kraussii* in a manuscript (see Endlicher 1843). Most likely, the collections were not all made on the same date. Krauss published the paper attributed to Hering (1844) after Hering's death, and the illustrations in this paper may have been made from material in

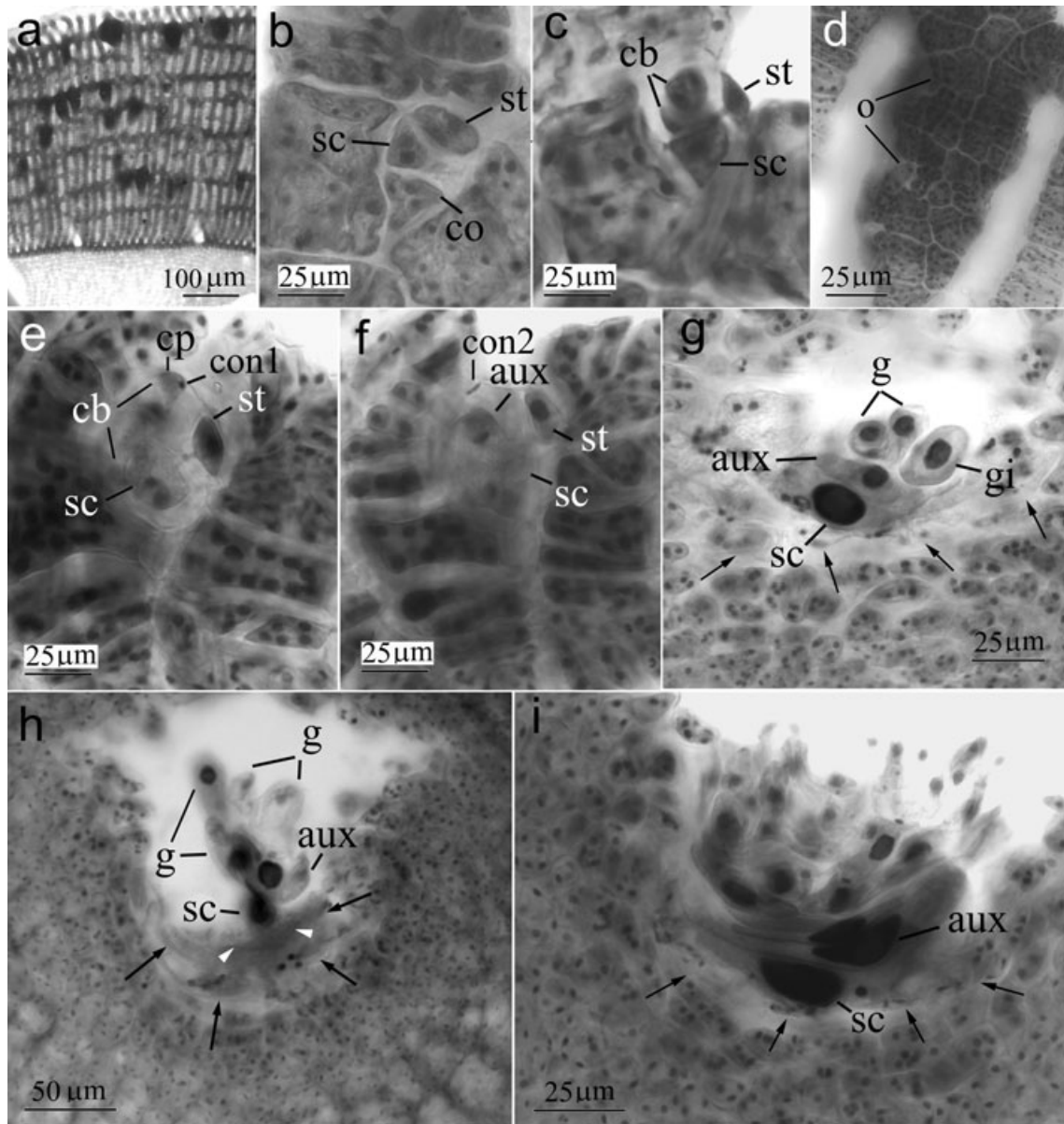


FIG. 2. *Martensia elegans* (Palm Beach 2005, Natal, South Africa). Procarp structure and early development of cystocarp. (a) Part of a network with cystocarps (darkly staining spots) at intersection of longitudinal lamellae and cross-connecting strands. (b) Young procarp showing cover cell initial (co) and supporting cell (sc) bearing a sterile cell (st). (c) Nearly mature procarp showing supporting cell (sc) bearing a sterile cell (st) and a carpogonial branch (cb). (d) Close-up of early formation of ostiole (o) and pericarps derived from surface cells of longitudinal lamellae adjacent to cross-connecting strands. (e) Postfertilization stage showing the first connecting cell (con1) cut off from a fertilized carpogonium (cp) terminating a carpogonial branch (cb), supporting cell (sc), and sterile cell (st). (f) A stage similar to that in (e), showing a second connecting cell (con2) cut off from a fertilized carpogonium and an auxiliary cell (aux) and sterile cell (st) borne on a supporting cell (sc). (g) Early postfertilization stage showing an auxiliary cell (aux) and gonimoblast initial (gi) cutting off a few gonimoblast cells (g). Note that the nucleus of the supporting cell (sc) is enlarged and darkly staining, and the floor cells (arrows) around the supporting cell have become vacuolate. (h) A later stage showing fusion of the floor cells (arrows) and that the cell wall of the supporting cell (sc) remains intact (arrowheads) separate from the auxiliary cell (aux) and gonimoblasts (g). (i) Young carposporophyte showing enlarged nuclei in the auxiliary cell (aux) and supporting cell (sc). Note that the supporting cell has not yet fused with the floor cells (arrows).

Krauss's possession at the time. No samples have been recovered that correspond to the habit drawings figured in Hering (1844, pl. vii, figs. 1–3).

*Type locality and distribution:* Port Natal (Durban), South Africa. Encountered commonly along the coast of Natal and the northern region of the Eastern Cape Province.

*Habitat and seasonality:* Collections were made from June through August and in December. Plants were epiphytic on coralline red algae or were attached on rock substrate.

*Specimens examined:* South Africa: (1) Natal Province: Reunion Rocks, The Bluff, Durban, coll. O. de Clerck, 17.vii.2005 (tetrasporic); Scottsburg, coll.

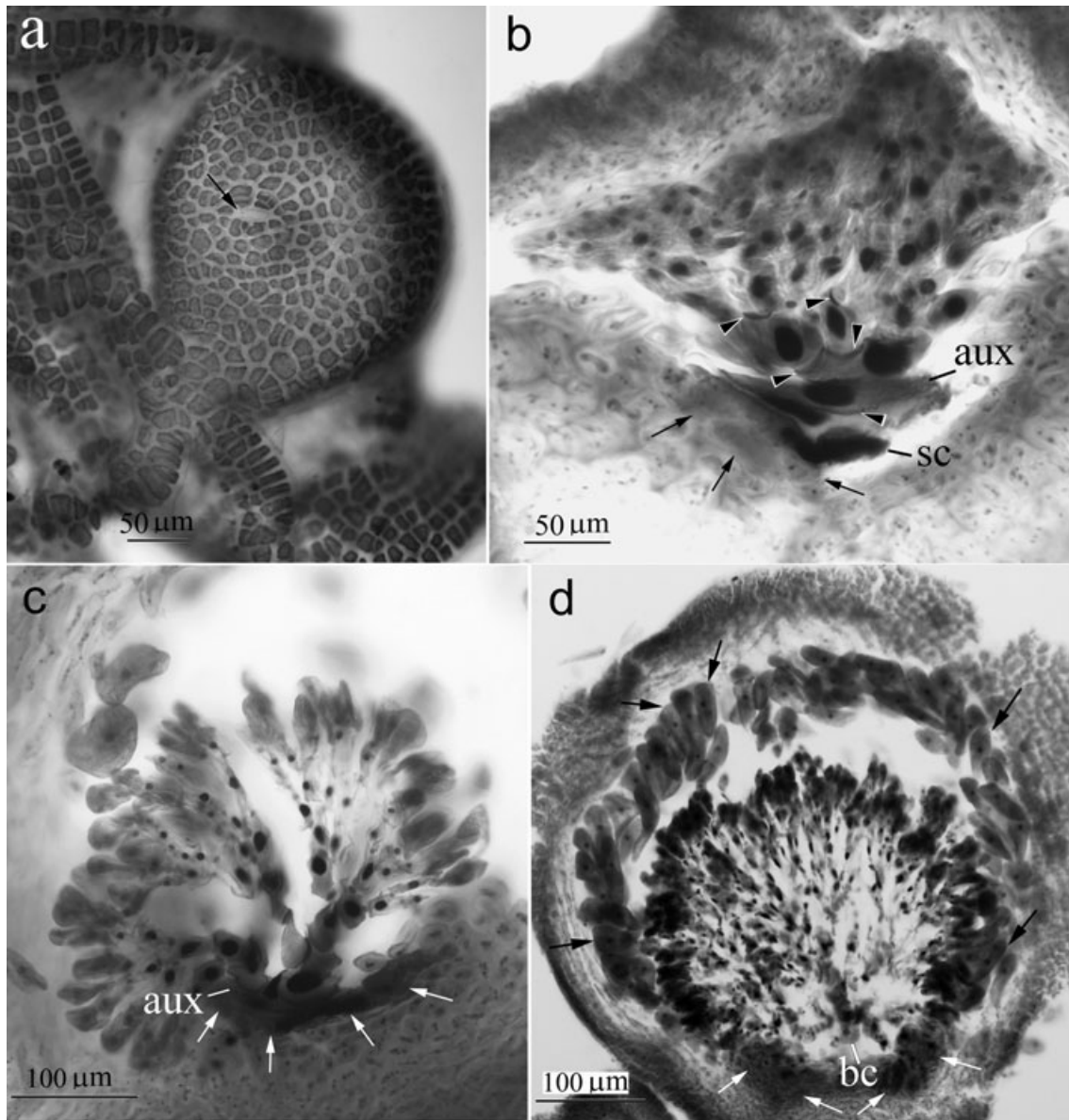


FIG. 3. *Martensia elegans* (Palm Beach 1993: b; 2005: a, c–d, Natal, South Africa). Cystocarp development. (a) Surface view of the pericarp and ostiole (arrow) of a young cystocarp. (b) Developing gonimoblasts showing subdichotomous and radial growth and broadened pit-connections (arrowheads) between the auxiliary cell (aux) and innermost gonimoblast cells. Note that the supporting cell (sc) has fused with floor cells (arrows). (c) Nearly mature carposporophyte, showing that the supporting cell is fully fused with the floor cells (arrows), whereas the auxiliary cell (aux) remains intact. (d) Cross-section through a fully developed cystocarp showing the basal fusion cell (bc) and darkly staining floor cells (white arrows) and the carposporangia (arrows).

O. de Clerck & F. Leliaert, 17.vi.2003 (tetrasporic, female); Palm Beach, coll. F. & M. Hommersand, 23.vii.1993 (female); Palm Beach, coll. S. Fredericq, 22.viii.2005 (tetrasporic, female, male); Umdlotti Beach, coll. G. Lambert & M. Hommersand, 25.vi.1983 (tetrasporic, female, male); Umdlotti Beach, coll. S. Fredericq, 19.viii.2005 (tetrasporic, female); Shelly Beach, coll. H. Verbruggen, 21.viii.2005 (tetrasporic). (2) Eastern Cape Province: Mzamba, south of Port Edward, coll. S. Fredericq, 21.viii.2005 (male, female, tetrasporic).

*Habit and vegetative morphology:* Thalli are attached to solid substrate by a discoid holdfast and short stipe 1–5 mm long that bears 1–4 (–6) fan-shaped flabellate blades 3–5 (–6) cm high and 2–7 cm wide (Fig. 1, a–c). The blades are pink to dark red or greenish when exposed to strong sunlight and consist of a membranous proximal part that ranges in thickness from one to six cell layers and a distal network composed of a single continuous band that extends over 10–60 (–70)% of the height of the blade surmounted by a narrow membranous margin

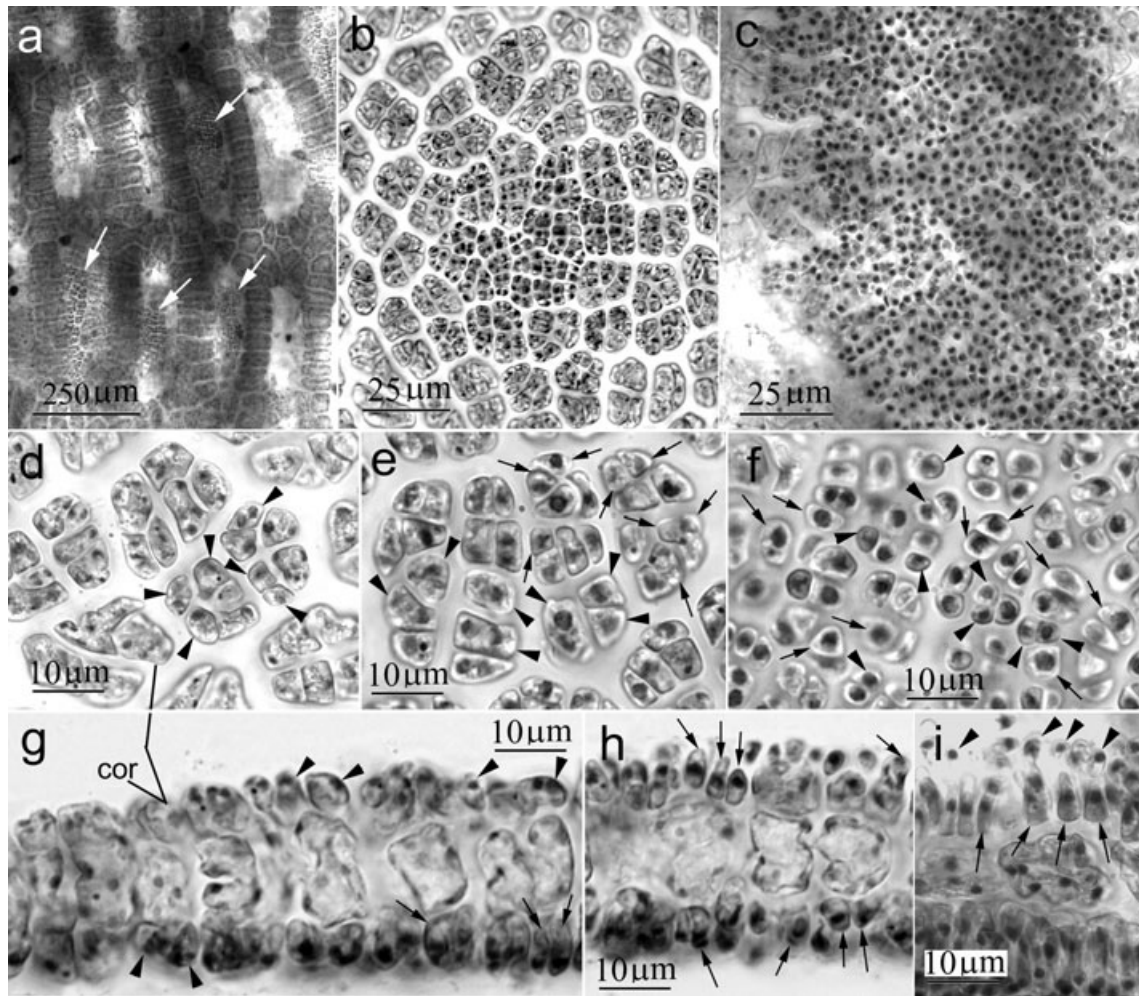


FIG. 4. *Martensia elegans* (Palm Beach 2005, Natal, South Africa). Male structures. (a) Spermatangial sori (arrows) on longitudinal lamellae. (b) Surface view of a maturing spermatangial sorus. (c) Surface view of aggregated mature spermatangial sori. (d) Surface view of spermatangial parent cell initials (arrowheads). (e) Surface view of spermatangial parent cell initials (arrowheads) and spermatangia (arrows). (f) Close-up of spermatangial parent cells (arrows) and spermatangia (arrowheads). (g) Cross-section through a male sorus showing spermatangial parent cells (arrows) and dividing fertile cortical cells (cor). (h) Cross-section through a male sorus showing immature spermatangial parent cells (arrows). (i) Cross-section through a male sorus showing mature spermatangial parent cells (arrows) and spermatangia (arrowheads).

(Fig. 1, a–c). Thallus margins are mostly entire but may occasionally be toothed.

Growth is diffuse due to the meristematic activity of multinucleate marginal cells and intercalary cell divisions (Fig. 1, d–h). The network is initiated from a row of transformed marginal cells that become vacuolate at their bases and densely filled with cytoplasm at their tips (Fig. 1d). Each cell cuts off an apical initial that divides transversely, followed by transverse division and acropetal maturation of the intercalary cells (Fig. 1, d and e). The uppermost cells become vacuolated and united by secondary pit-connections and undergo intercalary cell divisions to reform a membranous margin, and, at the same time, the subterminal darkly stained cells also divide transversely to initiate the intercalary longitudinal lamellae (Fig. 1, e and f). Cross-connecting strands are initiated bidirectionally from the edges

and inside the margins of the primary longitudinal lamellae (Fig. 1g) that meet in the space between the longitudinal lamellae where they unite by secondary pit-connections (Fig. 1h). Meanwhile, the basal cells of the longitudinal lamellae (Fig. 1f) divide longitudinally at first (Fig. 1f), followed by a succession of anticlinal cell divisions (Fig. 1g). Each cell contains many discoid plastids as seen in surface view, and numerous secondary pit-connections are formed between the cells of the membranous parts of the blade (Fig. 1i).

*Reproductive morphology.* Cystocarps are mostly formed along the edges of the longitudinal lamellae near the intersections of the longitudinal lamellae and cross-connecting strands (Fig. 2, a and d). 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 of the carpogonial

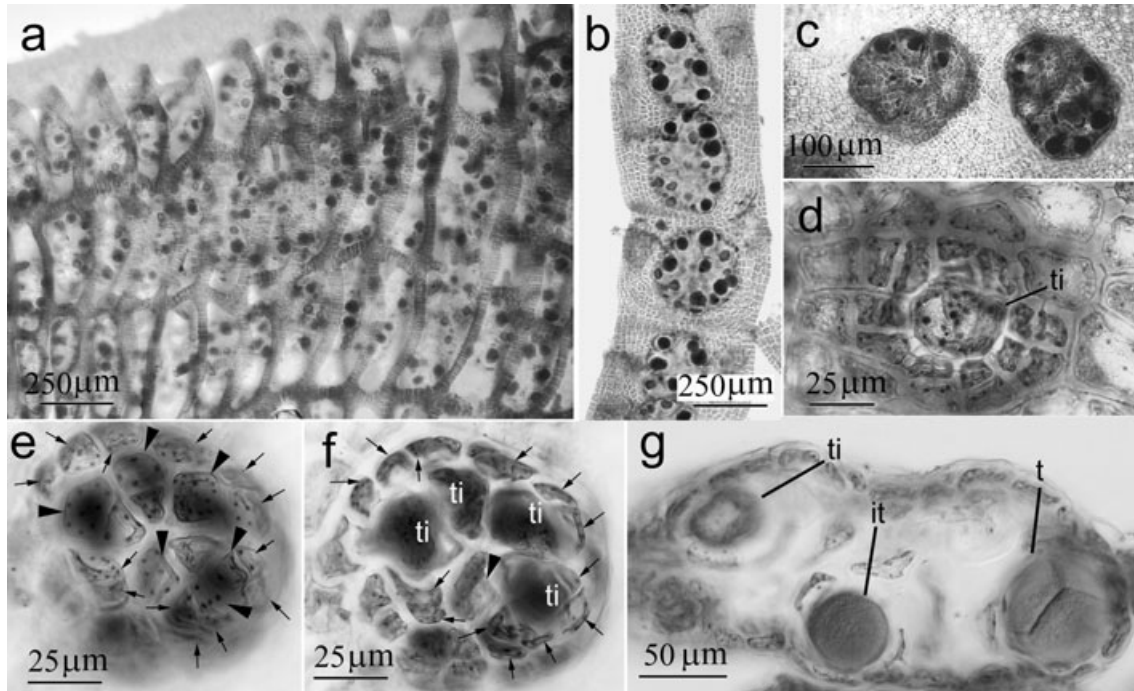


FIG. 5. *Martensia elegans* (Palm Beach 2005, Natal, South Africa). Tetrasporangial structures. (a) Tetrasporangial sori on network. (b) Close-up of tetrasporangial sori on a longitudinal lamella. (c) Close-up of tetrasporangial sori on membranous portion of thallus. (d) Surface view of a multinucleate tetrasporangial initial (ti). (e) Surface view of a young tetrasporangial sorus showing surface cells (arrowheads) above the tetrasporangial initials (not in focus) and neighboring cells (arrows). (f) View of the same tetrasporangial initials (ti) in the plane of focus. Arrowhead and arrows correspond to those cells shown in (e). (g) Cross-section of a longitudinal lamella showing a multinucleate tetrasporangial initial (ti), an immature tetrasporocyte (it), and a tetrahedrally divided tetrasporangium (t).

branch. The supporting cell first cuts off a sterile cell laterally (Fig. 2b) and then initiates the carpogonial branch. Procarps consist of a group of cover cells and a supporting cell bearing a lateral sterile cell and a slightly curved four-celled carpogonial branch (Fig. 2c). The majority of four-celled carpogonial branches observed had aborted. A fertilized carpogonium first cuts off a single connecting cell subterminally (Fig. 2e), then the supporting cell cuts off an auxiliary cell acropetally, and a second connecting cell is produced from the fertilized carpogonium at the same time (Fig. 2f). The pericarp is derived from the surface cells of the lamella surrounding the fertilized procarp by transverse and anticlinal cell divisions (Fig. 2d).

At an early stage of cystocarp development, the auxiliary cell cuts off a gonimoblast initial obliquely that, in turn, cuts off a row of gonimoblast cells anticlinally and obliquely (Fig. 2, g and h). Meanwhile, the nuclei in the supporting cell, the auxiliary cell, and the young gonimoblast cells enlarge and become densely staining, and the fertile central cell and its neighboring central cells lose most of their cytoplasmic contents (Fig. 2g) and unite to form a vacuolated fusion cell (Fig. 2h). As cystocarp development continues, the gonimoblast filaments branch subdichotomously and radially (Fig. 2i). By this stage, the surrounding cortical cells have formed a conspicuous pericarp with a central ostiole

(Fig. 3a). The nuclei of the supporting cell and auxiliary cell stain darkly and become greatly enlarged during early postfertilization stages (Fig. 2, g and i). During maturation, the pit-connections between the supporting cell and the auxiliary cell, and those between the inner gonimoblast cells broaden. At the same time, the enlarged nucleus of the supporting cell degenerates, and the vacuolated floor cells begin to fuse with the supporting cell to form a partly fused cellular mass (Fig. 3b). Later, this cellular mass fuses to form a darkly staining fusion cell (Fig. 3, c and d). The pit-connections broaden between the auxiliary cell and the primary gonimoblast cells, and between inner gonimoblast cells (Fig. 3b), and their enlarged nuclei remain distinct prior to maturation of the carposporangia (Fig. 3, b and c). Even after the carposporangia have fully matured, the shape of the auxiliary cell remains intact; however, the broadened pit-connections between the gonimoblast initial and some innermost gonimoblast cells may break down to form a branched fusion cell at the bases of the gonimoblast filaments (Fig. 3d).

Spermatangial sori are formed on primary longitudinal lamellae and are solitary or aggregated (Fig. 4a). Sometimes they are also formed on membranous blades. Spermatangial sori range from irregularly oblong to rectangular in shape (Fig. 4, b and c). Surface cells on both sides of membranous

blades or longitudinal lamellae (Fig. 4, d and g) divide anticlinally four to five times to form successively smaller cells (Fig. 4, e and h), which mature into spermatangial parent cells (Fig. 4, f and i). Each spermatangial parent cell cuts off one to two spermatangia (Fig. 4, f and i).

Tetrasporangial sori are produced primarily on longitudinal lamellae (Fig. 5a), are rounded to ovoid in shape, and are 150–300 by 100–250  $\mu\text{m}$  in diameter (Fig. 5b). Sometimes they are produced on membranous portions of the thallus where they measure 250–550 by 300–750  $\mu\text{m}$  in diameter (Fig. 5c). Tetrasporangial initials are transformed from multinucleate lamellar cells, or from surface cells in membranous portions of the thallus below the network (Fig. 5d). The tetrasporocytes (Fig. 5e) become covered by surface cells (Fig. 5, e and f) derived from neighboring cells (Fig. 5, e and f) in the course of their development, and each tetrasporocyte is transformed from a multinucleate to a uninucleate condition through nuclear degeneration, leaving behind a single functional nucleus (Fig. 5g). Mature tetrasporangia are tetrahedrally divided (Fig. 5g).

***Martensia natalensis*** S. M. Lin, Hommersand, Fredericq et De Clerck sp. nov. (Figs. 6, 7).

Thalli rosei vel rubri, flabelliformes, 1–2 cm alti, 1–2.5 cm lati, reticulo e vitta singula continua margine lobato membranaceo constanti; ponticuli transeuntes nulli in reticulis juvenibus, in reticulis maturis irregulariter dispositi, ponticulis obliquis 8–12 separati. Gametophyta dioica, isomorphica atque tetrasporophyta. Reticuli in plantis masculis ignoti, soris spermatangialibus in superficiebus ambabus laminarum fertilium membranacearum portatis. Procarpia cystocarpiaque intra reticulum facta in lobis marginalibus lamellarum longitudinalium, nec in membrana nec in margine thalli; procarpia constantia e grege cellularum tegentium et cellula sustinente cellulam unicam sterilem et ramum carpogonialem 4-cellulam ferenti; gonimoblasti ad apices facta, ramis radialibus vel subdichotomis; cellula sustinens, auxiliaris, et gonimoblasti initialis conjunctiones foveatas latiores et nucleos majores efferentes; cellulae solo cystocarpium vacuolatae factae, conjungentes cum cellula sustinente et cellulam fusionis basalem facientes. Sori tetrasporangiales 100–250  $\mu\text{m}$  in diametro, e cellulis superficialibus vel in lamellis longitudinalibus vel in membrano thalli orientes; tetrasporangia 75–80  $\mu\text{m}$  in diametro.

Thalli pink to red, fan-shaped, 1–2 cm high by 1–2.5 cm wide, the network consisting of a single continuous band with a lobed membranous margin; cross-connecting bridges absent in young networks and irregularly spaced, separated by 8–12 obliquely oriented bridges in mature networks. Gametophytes dioecious, isomorphic with the tetrasporophytes. Networks unknown in male plants, the spermatangial sori borne on both sides of fertile membranous blades. Procarps and cystocarps formed within the

network on the marginal lobes of longitudinal lamellae, not on the thallus membrane or thallus margin; procarps consisting of a group of cover cells and a supporting cell that bears a one-celled sterile cell and a four-celled carpogonial branch; gonimoblasts formed terminally, radially to subdichotomously branched; supporting cell, auxiliary cell, and gonimoblast initial developing broadened pit-connections and enlarged nuclei; cells in the floor of the cystocarp becoming vacuolated, uniting with the supporting cell to form a basal fusion cell. Tetrasporangial sori 100–250  $\mu\text{m}$  in diameter, originating from surface cells in longitudinal lamellae or in the thallus membrane; tetrasporangia 75–80  $\mu\text{m}$  in diameter.

*Type locality*: 9 Mile Reef, Sodwana Bay, Natal Province, South Africa (27°25'0.12' S, 32°43'50.16' E).

*Etymology*: “*natalensis*” refers to Natal, South Africa, where this new red alga was found.

*Holotype*: In GENT, no. KZN2168a, tetrasporophyte (Fig. 6a). Isotype in GENT, no. KZN 2168 b-d.

*Habitat and seasonality*: The collections were made in February, May through June, and August. Plants were epiphytic on coralline algae or *Champia*, or were attached on the reef to rock at the infralittoral fringe or in water 4–11 m deep.

*Specimens examined*: Natal Province, South Africa: 9 Mile Reef, Sodwana Bay, coll. O. De Clerck & S. Fredericq, 12.ii.2001 (tetrasporic); Scottsburgh, coll. O. De Clerck & F. Leliaert, 17.vi.2003 (female); Umdloti Beach, coll. S. Fredericq, 19.viii.2005 (tetrasporic, female, male); Isipingo Beach, coll. O. De Clerck & F. Leliaert, 16.vi.2003 (tetrasporic, female, male); Mission Rock, coll. O. De Clerck & F. Leliaert, 16.vi.2003 (silica gel material only).

*Habit and vegetative morphology*: Thalli are fan-shaped and flabellate, 1–2 cm high and 1–2.5 cm wide, and have a network consisting a single continuous band and a membranous margin that extends across 10%–50% of the blade (Fig. 6, a and b). The network is often seen only when thalli are bearing reproductive structures. Several plants are usually intermixed to form a mat, attached to solid substrate by multicellular, filamentous rhizoids, or are epiphytic on *Champia* or species of the Corallinaceae (Fig. 6b). Blades are bright red to pink, and margins of blades bear numerous lobes (Fig. 6, a and b).

Growth is diffuse by meristematic activity of multinucleate marginal and intercalary meristems, and a network is initiated from a row of transformed marginal cells. Cross-connecting filaments are not formed in young networks (Fig. 6c). The cells of cross-linking bridges are cut longitudinally from cells of longitudinal lamellae first (Fig. 6d), and the initials of the cross-linking bridges divide quickly by oblique cell divisions (Fig. 6e). Eight to 12 cross-linking bridges are produced in fully developed networks, and the basal cells of the longitudinal



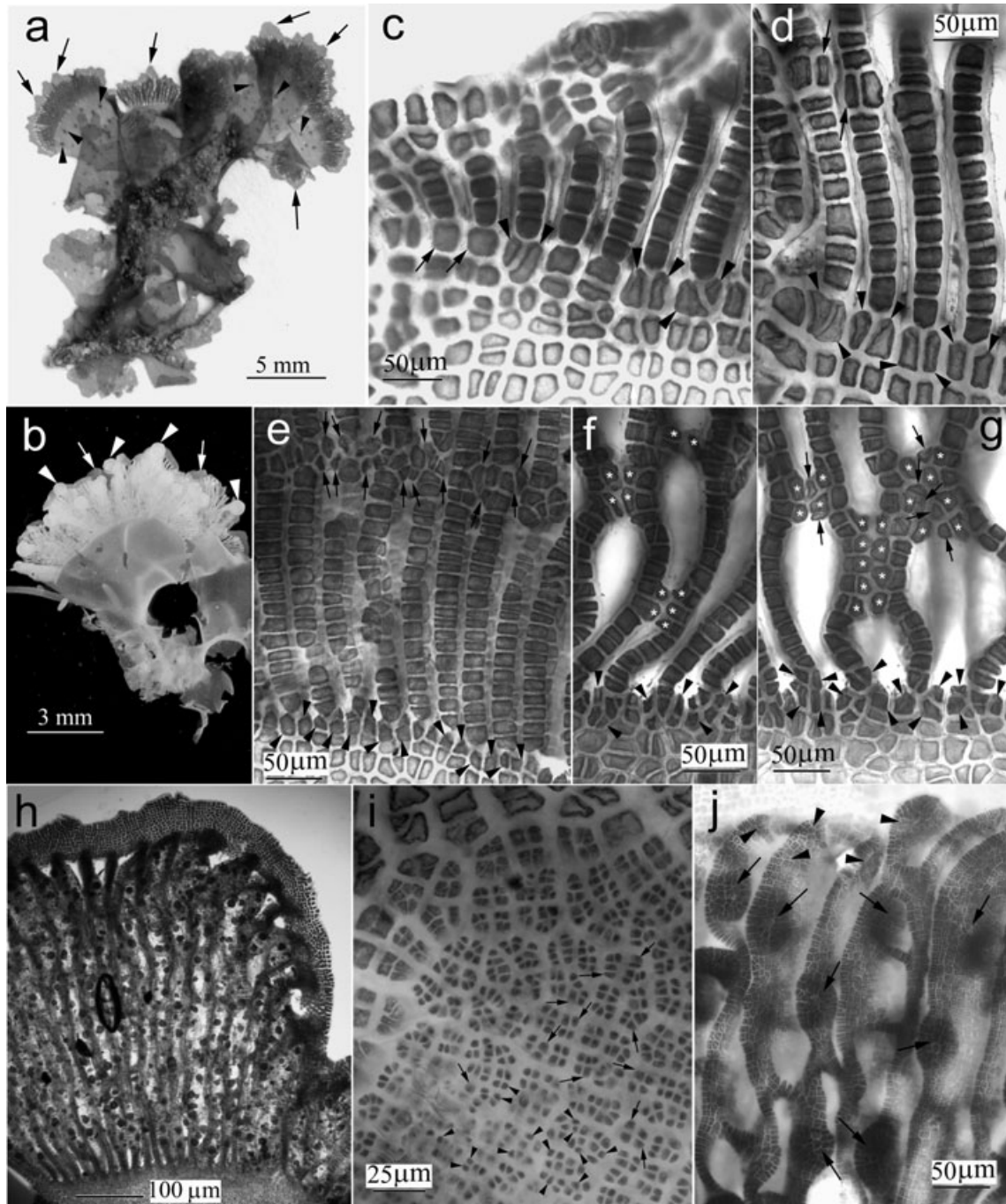


FIG. 6. *Martensia natalensis* sp. nov. (Natal, South Africa). Habit, vegetative and reproductive morphology. (a) Holotype, a tetrasporic plant with tetrasporangial sori (arrowheads) in the network and on membranous parts of the blade (9 Mile Reef, Sodwana Bay). Note that the lobed margins (arrows) are characteristic of this new species. (b) A female plant bearing cystocarps (arrowheads) and marginal lobes (arrows). (c) Early stage of network formation, noting basal cells (arrows) of longitudinal lamellae undergoing longitudinal and oblique cell divisions (arrowheads). (d) Early formation of cross-link bridge formation by longitudinal cell divisions (arrows) of longitudinal lamellae and longitudinal and oblique cell divisions (arrowheads) of basal cells. (e) Later stage of cross-link bridge formation (arrows) and transverse and oblique cell divisions of basal cells (arrowheads). (f) Basal portion of older network showing early formation of cross-bridge cell initials (asterisks) and basal cells (arrowheads). (g) Basal portion of old network showing cross-bridge cell initials (asterisks) cutting off small connecting cells (arrows). Note that the basal cells of longitudinal lamellae (arrowheads) may not divide or may divide obliquely once. (h) Close-up of a network showing tetrasporangial sori on longitudinal lamellae. (i) Surface view of a spermatangial sorus on membranous part of thallus showing spermatangial parent cells (arrows) and spermatangia (arrowheads). (j) Part of network of a female plant showing developing cystocarps (arrows) and procarp-bearing lobes (arrowheads) originating from the edges of longitudinal lamellae.

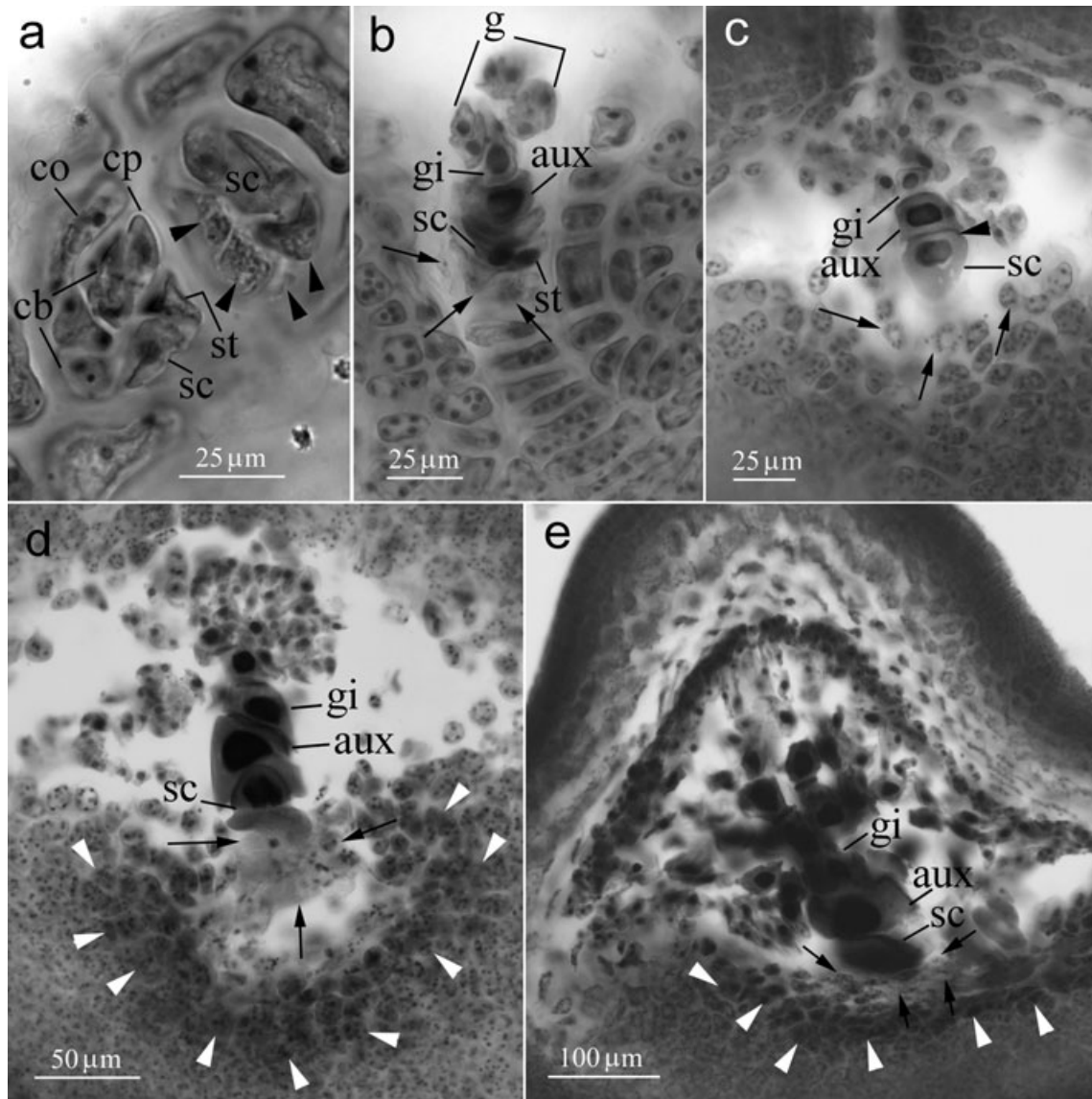


FIG. 7. *Martensia natalensis* sp. nov. (Umdloti, Natal, South Africa). Procarp structure and carposporophyte development. (a) Two procarps: the one on the left-hand side showing a group of cover cells (co) and a supporting cell (sc) bearing a carpogonial branch (cb) with a functional carpogonium (cp) and a sterile cell (st), and the one on the right-hand side showing the supporting cell (sc) bearing a degenerating carpogonial branch (arrowheads). (b) Early postfertilization stage showing the auxiliary cell (aux) and gonimoblast initial (gi) cutting off a cluster of gonimoblast cells (g). Note that the supporting cell (sc) and sterile cell (st) remain intact and that the floor cells (arrows) around the supporting cell have become vacuolated. (c) A later stage showing the supporting cell (sc) surrounded by the expanded floor cells (arrows), the broadened pit-connection (arrowhead) between the supporting cell and the auxiliary cell (aux) and their enlarged nuclei, and the gonimoblast initial (gi) bearing radially branched gonimoblasts. (d) Young carposporophyte showing subdi- chotomous and radial growth of the gonimoblast filaments. Note that the supporting cell (sc) has fused with the neighboring floor cells (arrows) while maintaining its cellular shape, and that the floor cells surrounding the fusion cell (arrowheads) stain darkly. (e) An immature carposporophyte showing darkly staining thickened floor cells (arrowheads) and the vacuolated inner floor cells (arrows). Note that the gonimoblast initial (gi) and the auxiliary cell (aux) remain intact, but that the nucleus of the supporting cell (sc) has disintegrated.

lamellae may or may not divide longitudinally or obliquely once or twice (Fig. 6, f and g).

**Reproductive morphology:** Gametophytes are dioecious and similar to the tetrasporophytes (isomorphic). Tetrasporic (8–16 mm in height, Fig. 6a) and male plants (10–18 mm in height) are usually smaller than female gametophytes (12–20 mm in height, Fig. 6b). Tetrasporocyte initials originate from

ordinary surface cells of longitudinal lamellae (Fig. 6h) or membranous parts of the thallus (Fig. 6a). Tetrasporangial sori are minute, irregularly circular in shape, 100–250  $\mu\text{m}$  in diameter. Mature tetrasporangia are tetrahedrally divided and 70–85  $\mu\text{m}$  in diameter. Networks were not observed in male plants. Spermatangial sori are formed on both sides of fertile membranous blades and are

minute and irregularly discoid to plate-like in shape (Fig. 6i). Surface cells of membranous parts of blades divide anticlinally four to five times to form successively smaller cells, which mature into spermatangial parent cells (Fig. 6i), and each parent cell divides obliquely to cut off one to two spermatangia (Fig. 6i).

Procarp-bearing lobes (Fig. 6j) are abundant along the margins of longitudinal lamellae within the network, in which the cystocarps develop (Fig. 6j). Procarps are initiated from surface cells of the marginal lobes of longitudinal lamellae. The fertile central cell cuts off two pericentral cells, the first being the cover cell initial, and the second the supporting cell, which in turn cuts off a one-celled sterile cell and a carpogonial branch (Fig. 7a). Aborted procarps were often observed next to a functional procarp (Fig. 7a).

Early postfertilization stages were not seen in the specimens examined. Young gonimoblast cells are cut off terminally in clusters from the gonimoblast initial in early stages of gonimoblast development. The supporting cell, sterile cell, and the auxiliary cell stain darkly at this stage, and the gonimoblast filaments branch subdichotomously and radially (Fig. 7b). The nuclei of the supporting cell, sterile cell, and auxiliary cell enlarge significantly, and the floor cells near the supporting cell become vacuolated (Fig. 7c). Pericarp cells are derived from the cells surrounding fertilized procarp, and ostioles are off-center at the tops of cystocarps. As cystocarp development continues, the floor cells near the supporting cell lose most of their cytoplasmic contents and unite to form a vacuolated fusion cell, and the pit-connection between the supporting cell and the fertile central cell breaks down (Fig. 7d). Pit-connections between the supporting cell, auxiliary cell, and the inner gonimoblast cells broaden; however, these cells do not fuse before differentiating and producing the carposporangia (Fig. 7e). Fully mature carposporophytes were not seen in our material.

**Molecular analysis:** A set of 23 *rbcl* sequences within the genus *Martensia* plus five other members of the Nitophyloideae (*Augophyllum*, *Calonitophyllum*, *Nitophyllum*, and *Valeriemaya*), which served as the outgroup, were selected for the analysis. Five *rbcl* sequences of *M. natalensis* and four of *M. elegans* were newly generated in this study (see Fig. 8). The *rbcl* alignment consisted of 1,467 base pairs (bp), but because information was missing for the 5' ends of many sequences, the first 60 bp were excluded from the analyses. The final data matrix was restricted to 1,407 bp. MP analysis resulted in two most-parsimonious trees with tree length of 697 steps, CI = 0.6729, and RI = 0.8484. There were 294 informative characters out of 1,407 included sites. Tree topologies generated under ML and Bayesian inference (BI) were highly congruent with the MP trees, differing only in the position of *M. martensii* as well as the *M. speciosa*/*M. formosana* clade.

*M. martensii* occupies a sister relationship with respect to the *M. fragilis*/*M. lewisiae* clade under MP, while its position is unresolved under ML and BI. Likewise, ML and BI failed to resolve the relationship of the *M. speciosa*/*M. formosana* clade, resulting in a polytomy. The respective taxa come out as a grade under MP. Only the consensus tree of the Bayesian analysis is shown (Fig. 8). All 21 taxa of *Martensia* species within the tribe Martensieae included in the analysis resulted in a monophyletic clade with strong bootstrap support (BP = 100). *M. elegans* and *M. natalensis* were distantly related with 12.2% of *rbcl* sequence divergence (Kimura 2 parameter distance) and were situated in two separate subclades, referred to as subgenus *Martensia* and subgenus *Mesotrema*.

#### DISCUSSION

Our present understanding of *Martensia* is primarily owing to the work of Svedelius (1908) who investigated the development and construction of the network that characterizes the genus and typically contains the reproductive structures at maturity. Svedelius established that prior to network formation the thallus grows by a marginal meristem accompanied by transverse, longitudinal, and oblique divisions of the intercalary cells to form a complex single-layered membrane, much as in *Nitophyllum*. At the time of network formation, the basal cells of participating cell rows remain undivided while the apical and intercalary cells continue dividing and form filaments that are transformed into lamellae in which lateral growth is directed perpendicular to the orientation of the original thallus. Network formation is completed by the production of multicellular cylindrical cross-connecting strands that link adjacent perpendicular lamellae. These strands may be produced in two directions and meet and fuse in the space between, or they may develop in one direction only and link to the lamella on the opposite side. The marginal and basal meristems and membranes are reformed as the network develops. Svedelius also concluded that a tetrasporangium is initiated from a multinucleate cell in *Martensia*, which undergoes nuclear degeneration giving rise to a single remaining nucleus that divides twice to produce the four nuclei of the tetraspores. These studies of tetrasporangium development in *Martensia* anticipated an investigation of the same process in *Nitophyllum punctatum* in which Svedelius (1914) coupled nuclear degeneration with meiosis in the residual nucleus.

Vegetative, male, female, and tetrasporangial development was investigated by Svedelius (1908) in material of *M. fragilis*, which he had collected at several localities in Sri Lanka. His work was supplemented by studies on herbarium specimens of *M. elegans*, *M. pavonia*, and *M. flabelliformis* with additional notes on *M. australis*, *M. denticulata*, and

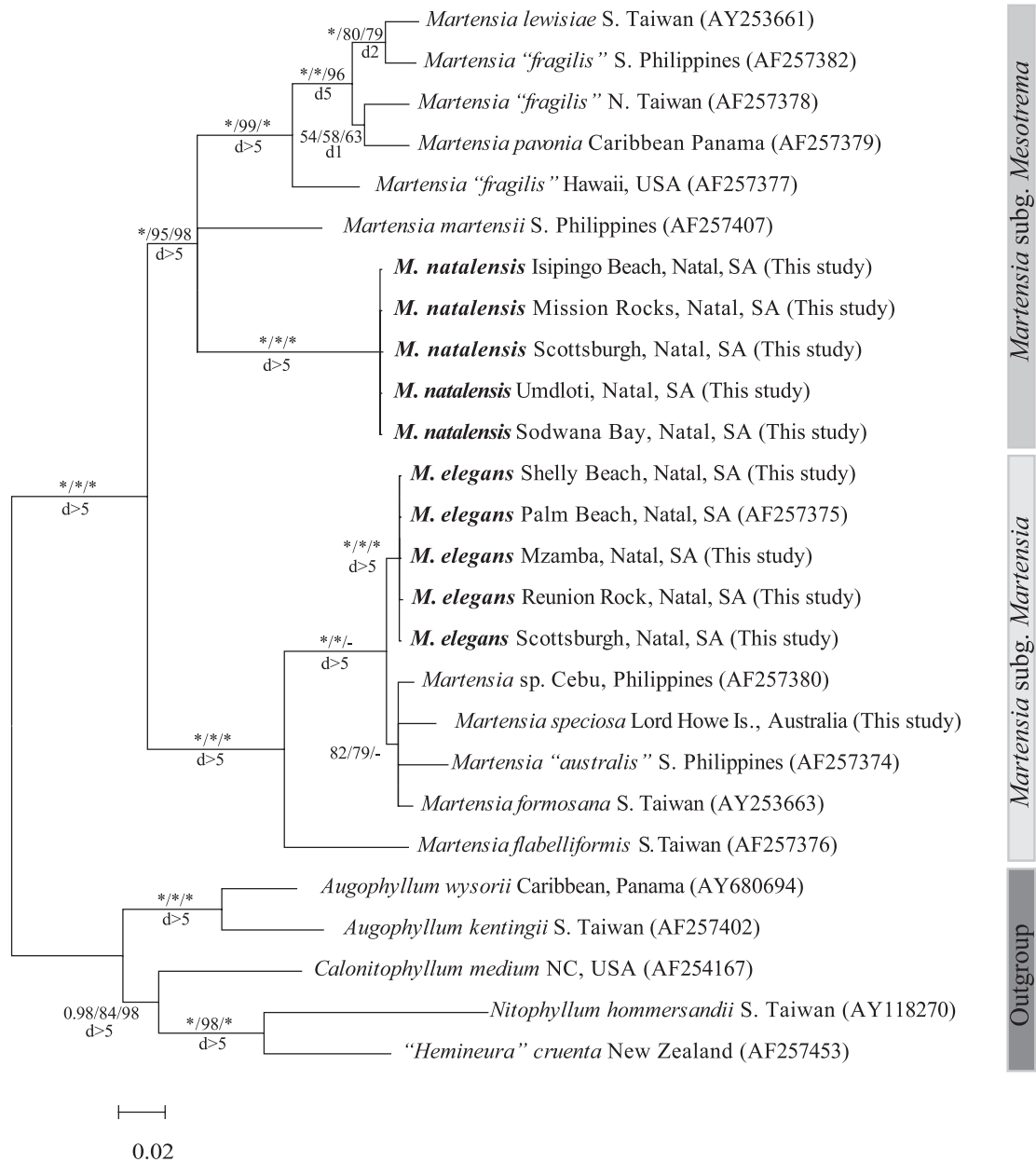


FIG. 8. Consensus tree of the genus *Martensia* resulting from a Bayesian analysis of the *rbL* gene, using a selection of the related nitophylloidean genera as outgroup taxa. Numbers above branches are Bayesian posterior probabilities, ML and MP bootstrap values, respectively; decay indices are shown below the nodes. An asterisk indicates support values of 100 (BS) or 1.0 (PP).

*M. speciosa*. Svedelius appears to have relied mainly on his own concepts of these species, which did not rely on type material. This is particularly evident in the case of *M. elegans*. As Svedelius pointed out, he based most of his observations of *M. elegans* on specimens collected in parts of the Indo-Malaysian Archipelago. Prof. O. Nordstedt provided Svedelius with photographs from the Agardh herbarium at Lund, including putative South African material. However, a reexamination of the cystocarpic plant (presumably from the Cape of Good Hope, South Africa, Hb. Agardh 36272) pictured by Svedelius

(1908, p. 28, fig. 31) proved to be an unlocalized specimen labeled *M. elegans* Her. situated next to a specimen (Hb. Agardh 36273) labeled Harvey Austral. algae, which it resembled. Millar (1990) examined the lectotype of *M. fragilis* Harvey (1854) from Ceylon and specimens distributed under that name by Harvey in his Alg. Ceylon Excic. #5. Some of these were multiple banded, unlike the specimens described by Svedelius. Although we failed to locate the material of *M. fragilis* collected by Svedelius and were unable to obtain new *Martensia* material from Sri Lanka, it is likely that the Svedelius

(1908) concepts of *Martensia* species were based on a mixture of specimens from different localities.

Thalli of *M. elegans* from Natal, South Africa, are characterized by having a thick, membranous basal blade surmounted by a single banded network and a single thin, smooth, membranous margin. Its size ranges from 3 to 5 cm in height. Plants of *Martensia* outside of South Africa that fit this general description have been treated as *M. elegans* (Agardh 1863, Svedelius 1908, Okamura 1909, Millar 1990, N'Yeurt et al. 1996); however, our recent analyses of *rbcl* sequences (Lin et al. 2001b, 2004a, this study, and S. M. Lin unpublished data) of a series of collections of *Martensia* species from the Indo-West Pacific Ocean suggest that *M. elegans* is an endemic species occurring only in South Africa. Clarifying the records of *M. elegans*, *M. fragilis*, and *M. australis* from the northern West Pacific Ocean (i.e., Taiwan, Korea, Japan; see Lin et al. 2004a, Lee 2006) is an ongoing project by the first author and her research associates.

A second, smaller species of *Martensia* that has a thinner network, *M. natalensis* sp. nov., 1–2 cm high, is also common in KwaZulu-Natal, South Africa. Separation of the two South African species is confirmed based on *rbcl* sequence analysis (Fig. 8). *M. elegans* was shown by Lin et al. (2004a) to form a clade that also contained *M. formosana* and *M. flabelliformis* from Taiwan, and *M. "australis"* from the southern Philippines. To this clade, we add an undescribed species from Cebu, Philippines, and *M. speciosa* from Lord Howe I., Australia (Fig. 8). *M. natalensis* belongs to the second clade designated the "*fragilis*" clade in Lin et al. (2004a), which also contained *M. lewisiae* (Taiwan), *M. "fragilis"* (S. Philippines, Taiwan and Hawaii), *M. pavonia* from Caribbean Panama, and *M. martensii* from S. Philippines, a species that lacks a network. *M. natalensis* occupies a basal position in this clade and is well separated from all the other species.

Having examined material representative of the type species of *Martensia*, *M. elegans*, from South Africa and a new species from Natal, *M. natalensis*, that is strongly supported in the clade that includes *M. pavonia* and other "*fragilis*"-like members, we conclude that the two strongly supported phylogenetic clades should be recognized at the subgeneric level as subgenus *Martensia* to contain the type species, *M. elegans* C. Hering, and related species and subgenus *Mesotrema* (J. Agardh) De Toni to contain *M. pavonia* (J. Agardh) J. Agardh and related species. At the present time, the two subgenera are separated morphologically primarily by the position of the procarps and cystocarps: at the intersections of longitudinal lamellae and cross-connecting strands in subgenus *Martensia* (see Fig. 2, a–b, in this study) and along the lobed edges of the longitudinal lamellae in subgenus *Mesotrema* (see Fig. 6j in this study). The subgenus *Martensia* procarp characterizes *M. elegans*, *M. formosana* (Lin et al. 2004a, figs,

39 and 40), *M. flabelliformis* from Taiwan and New Caledonia, and *M. "australis"* from southern Philippines and southeastern Australia (S. M. Lin unpublished observations). The *Mesotrema* procarp borne on the lobed edges of the lamellae or the thallus margin characterizes *M. natalensis*, *M. lewisiae* from Taiwan (Lin et al. 2004a, fig. 14), three entities referred to *M. "fragilis"* (Taiwan, S. Philippines, Hawaii), *M. martensii* from S. Philippines (Lin et al. 2001c, fig. 1) in which the cystocarps are formed along the blade margins, and probably also *M. pavonia* from the Caribbean Sea. In addition to procarp and cystocarp position, the precise pattern of network formation and the details of carposporophyte development may also differ in the two subgenera. The diagnostic value of these characters and the distribution of characters among the subgenera and species of *Martensia* must await further study and the completion of a planned monographic and biogeographic investigation of the *Martensia* species of the Indo-West Pacific Ocean (Lin 2007).

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### Supplementary Material

The following supplementary material is available for this article:

**Table S1.** List of species of *Martensia* used in *rbcl* analysis and accession numbers in GenBank.

**Appendix S1.** Other historical material.

This material is available as part of the online article.

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