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Neotypification and Taxonomic Status of *Opephyllum martensii* Schmitz *in* Schmitz *et* Hauptfleisch (Delesseriaceae, Rhodophyta) from Zamboanga, Southern Philippines

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The monotypic delesseriaceous genus *Opephyllum* was created by Schmitz in Schmitz and Hauptfleisch for *O. martensii*, represented by a single collection from Zamboanga on the southwestern tip of Mindanao in the southern Philippines. As the type specimen or collection is no longer in existence, we are neotypifying the taxon based on recent topotype collections made in the spring of 1998. After comparison of this material with species of *Martensia* from the Indo-Pacific region and Caribbean Sea based on morphological evidence and sequence analysis of chloroplast-encoded *rbc*L and the nuclear-encoded large subunit ribosomal DNA gene (LSU rDNA), we conclude that *Opephyllum* is not generically distinct from *Martensia* despite its lack of the reticulate fenestrations that characterize fronds of every other species of the latter. We therefore propose the new combination *Martensia martensii* (Schmitz *in* Schmitz *et* Hauptfleisch) Lin, Fredericq *et* Liao for this rare member of the tribe Martensieae, subfamily Nitophylloideae.

Introduction

The monotypic genus *Opephyllum*, based on *O. martensii*, was described by Schmitz in Schmitz and Hauptfleisch (1897) for plants collected by Martens from Zamboanga, Philippines, and has been placed in the Martensia Group by several authors (Kylin 1956, Papenfuss 1962, Wynne 1983, 1996). However, no-one has seen this alga since Schmitz described it. The etymology of *Opephyllum* means 'open leaf', probably a reference to the perforations occurring in the mature blade. Because reproductive structures and further collections were unknown and no illustrations of habit or morphology had ever been given, Papenfuss (1962) regarded *Opephyllum* as one of the major taxonomic problems still to be resolved in the Rhodophyta, although he foreshadowed that '…to judge from Schmitz's description, it seems likely that *Opephyllum* will be found to be congeneric with *Martensia* Hering…'.

Although authentic voucher material of Schmitz's species apparently no longer exists, having been most likely destroyed along with the Berlin Museum during the Second World War, our recent topotype collections fit Schmitz's description of blades that are similar in morphology to the proximal, non-reticulate portions of several *Martensia* species. In addition, our material includes male and female gametophytes and tetrasporophytes which permit accurate comparison with *Martensia* and distinction of the species from superficially similar blade-like Nitophylloideae, such as belonging to the genus *Nitophyllum* itself.

Materials and Methods

Collections of *Opephyllum martensii*, attached to rocks at 1–10 m depth, were made by SCUBA diving and snorkeling on 27 April and 1 May 1998 by the first author at La Vista Del Mar, Upper Calarian, Zamboanga (122°02′ E; 6°55′ N). Specimens for morphological studies were fixed in 10% Formalin/seawater. Nine individual thalli, the Neotype (Voucher number SML 1998-4-27-1-2) and duplicate topotype specimens (voucher numbers SML 1998-4-27-1-3, SML 1998-5-1-1-3) are housed as herbarium sheets or liquid-preserved in 5% Formalin/seawater in the Herbarium of the University of Louisiana at Lafayette (LAF) and the National Museum of Natural History (US). Herbarium abbreviations follow Holmgren *et al.* (1990).

Hand sections were stained in 1% aqueous aniline blue/Karo™ syrup/water (5:20:75) preserved with a few drops of phenol, or were treated with aceto-iron-hematoxylin-chloral hydrate (Wittman 1965) and mounted in 50% Hoyer's mounting medium (Lin and Kraft 1999). Photographs were taken on an Olympus BX60 Photomicroscope (Olympus, Melville, NY, USA) with a Polaroid DMC Ie digital camera (Polaroid Inc., Cambridge, MA, USA). Habits of specimens were scanned with a Microtek Scanmaker III (Microtek, Redonda Beach, CA, USA). Digital images were

edited and assembled on plates using Photoshop 4.0. Photographic plates were printed on an Epson Stylus Color 900 inkjet printer (Epson, Tokyo, Japan).

Silica gel-dried specimens and extracted DNA samples were deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at −20 °C. Vouchers selected for DNA sequence analysis are listed in Table I together with their GenBank accession numbers. The DNA samples were prepared using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The addition of 0.5×10^{-3} to 1×10^{-3} mg of proteinase K to each extraction when grinding the samples using the Qiagen Minikit often improved the polymerase chain reaction (PCR) yield (Hughey and Hommersand 1999). The genes selected were chloroplast-encoded *rbc*L and the nuclear-encoded large-subunit ribosomal DNA gene (LSU rDNA). Primers used and protocols for gene amplification and DNA sequencing are as described in Lin *et al.* (2001). The generated sequence data were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. The *rbc*L data set was aligned manually, whereas the LSU rDNA data set alignment was done first by using the online program ClustalW 1.8, and then this alignment was revised manually. Phylogenetic analyses were performed using the Maximum Parsimony algorithms available in the computer program PAUP (v.4.0*: Swofford 2000). Parsimony searches were done on each data set under the criterion of equal weights for all substitutions. Consistency (CI) and retention (RI) indices (Kluge and Farris 1989) were calculated excluding uninformative characters. Support for nodes was assessed by

Table I. List of species sequenced in this study and *rbc*L† and LSU rDNA‡ accession numbers in GenBank.

Species	Collection information	GenBank accession number
Calonitophyllum medium (Hoyt) Aregood	Radio Island, Bogue Sound, Carteret Co., North Carolina, USA; coll. M. Deals, 24.iv.94.	† AF254167, 97%; ‡ AF259413, 100%
Delesseria sanguinea (Hudson) Lamouroux	Newcastle, Co. Down, N. Ireland, United Kingdom; coll. C. Maggs, 15.iii.99.	† AF254182, 86%; ‡ AF259426, 68%
"*Hemineura*" *cruenta* Harvey	D1, Vailavae, New Zealand; coll. W. Nelson, 24.xi.94.	† AF257453, 98%; ‡ AF259486, 100%
Martensia australis Harvey	Little Santa Cruz Is., Zamboanga City, Philippines; coll. S.-M. Lin 28.iv.98.	† AF257374, 94%; ‡ AF259446, 100%
Martensia elegans Hering	Palm Beach, Natal Province, South Africa; coll. F. & M. Hommersand, 23.vii.93.	† AF257375, 98%; ‡ AF259447, 100%
Martensia fragilis Harvey	Yu-liu, Taipei Co., Taiwan; coll. S. Fredericq & S.-M. Lin, 7.vii.94.	† AF257378, 81%; ‡ AF259449, 99%
Martensia fragilis Harvey	Chiraman's Hat, Kameohe' Bay, Oahu, Hawaii, USA; coll. K. Cole, 29.v.98.	† AF257377, 85%; ‡ AF259448, 99%
Martensia pavonia (C. Agardh) J. Agardh	Cayos Zapatilla, Bocas del Toro, Caribbean Sea, Panama; coll. B. Wysor, 21.x.99.	† AF257379, 91%; ‡ AF259451, 77%
Martensia sp. 1.	Tambuli, Cebu, Philippines; coll. S.-M. Lin, 18.iv.98.	† AF257380, 80%; ‡ AF259445, 99%
Martensia sp. 2.	Little Santa Cruz Is., Zamboanga City, Philippines; coll. S.-M. Lin, 28.iv.98.	† AF257382, 99%; ‡ AF259452, 100%
"*Neomartensia*" *flabelliformis* (Harvey *ex* J. Agardh) Yoshida *et* Mikami	Wan Li Dong, Kenting National Park, Pingtung co., Taiwan; coll. S.-M. Lin & Q.-M. Lan, 23.xii.96.	† AF257376, 80%; ‡ AF259450, 60%
Nitophyllum punctatum (Stackhouse) Greville	Asturias, Spain; coll. C. Maggs, 5.iii.99.	† AF257402, 97%; ‡ AF259459, 95%
Opephyllum martensii Schmitz *in* Schmitz *et* Hauptfleisch	La Vista Del Mar, Upper Calarian, Zamboanga, Philippines; coll. S.-M. Lin, 1.v.98.	† AF257407, 93%; ‡ AF259461, 100%
Phycodrys rubens (Linnaeus) Batters	West Angle Bay, Pembrokeshire, United Kingdom; coll. F. & M. Hommersand, 22.vii.97.	† AF257429, 95%; ‡ AF259470, 100%
Polyneuropsis stolonifera Wynne, McBride *et* West.	North Jenner Beach, Sonoma Co., California, USA; coll. C. Kjeldsen, 14.viii.90.	† AF257439, 93%; ‡ AF259476, 55%

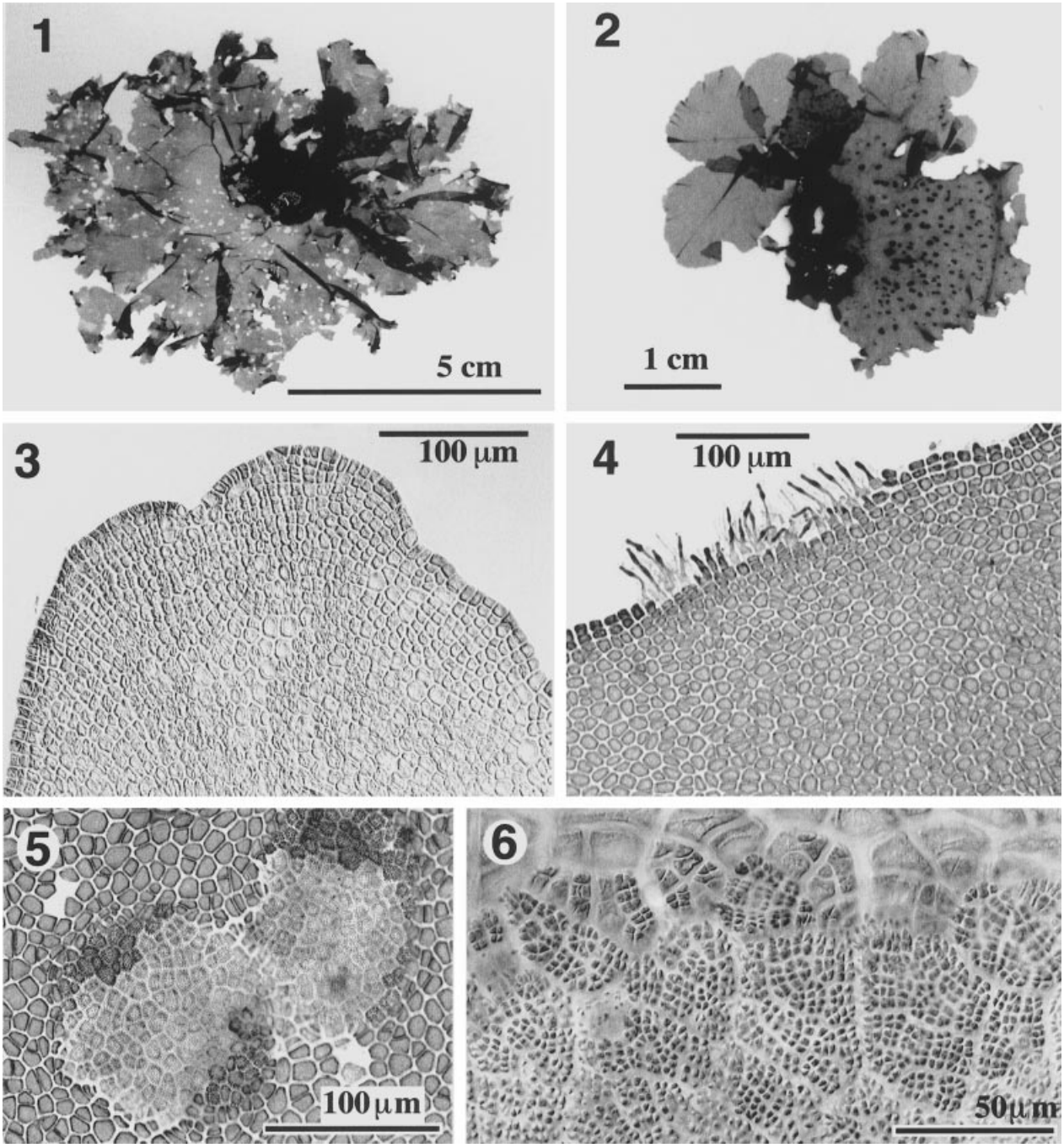
The number after the accession number is the percentage of the sequenced fragment of the gene.

calculating 1000 bootstrap resamplings of the heuristic searches based on random stepwise additions, MULPARS and TBR.

Results

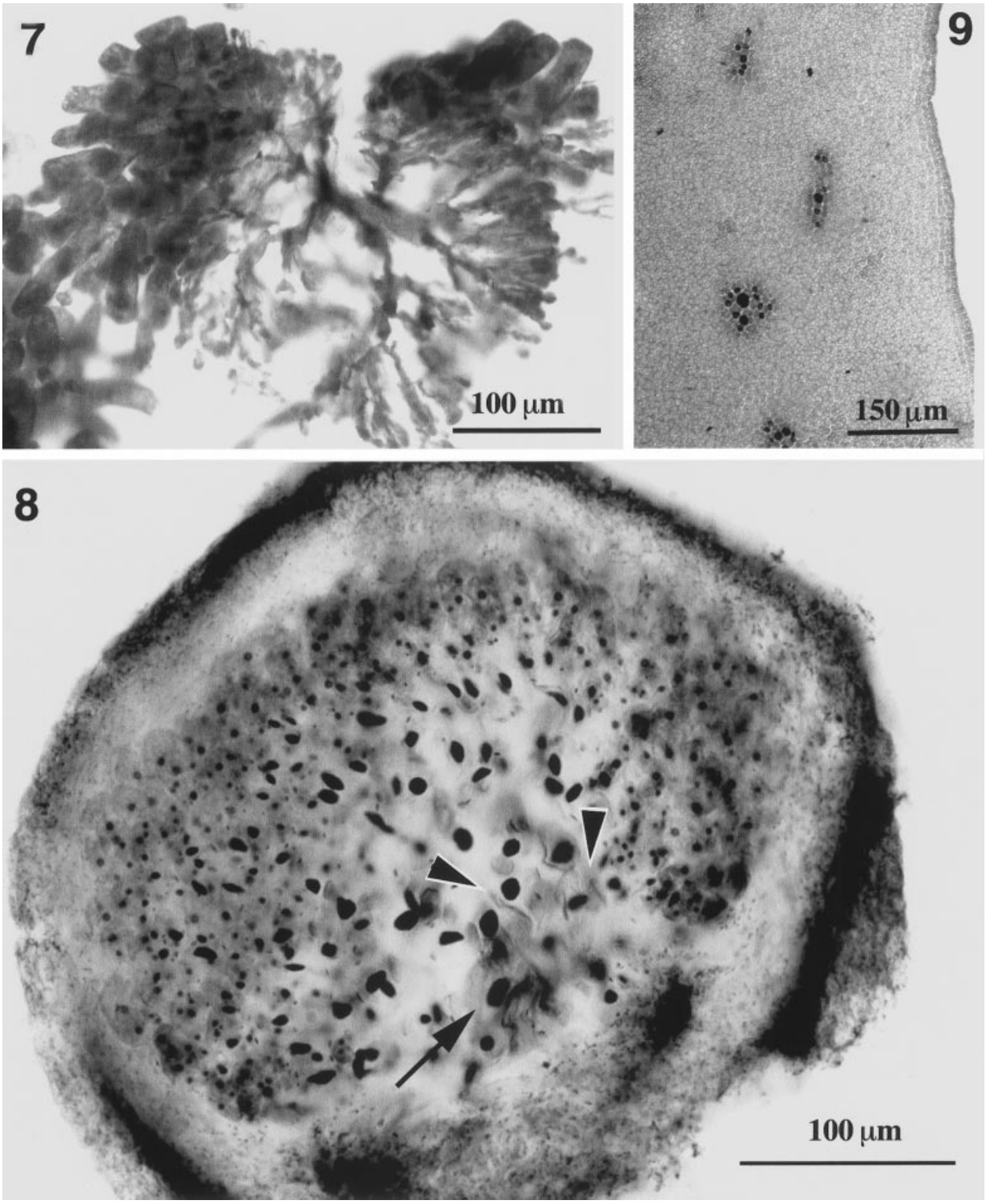
Vegetative structure

The thallus is erect to semi-prostrate, membranous, lacking macroscopic and microscopic veins, and reddish to pink in color. The margins are smooth, entire, lacerated (Fig. 1) or give rise to several partly overlapping rounded lobes (Fig. 2) on older fronds with isolated patches of unicellular rhizoids (Fig. 4) that secondarily attach to the substratum. Fronds are 5−10 cm in length by 3−10 cm in width, and arise singly and probably directly (without stipe or apophysis) from a discoid holdfast, although there are no basal parts on our material. Blades grow initially by



Figs 1−6. *Martensia martensii* (Schmitz *in* Schmitz et Hauptfleisch) Lin, Fredericq *et* Liao, comb. nov. (La Vista del Mar, Zamboanga, Philippines).
Fig. 1. Neotype, cystocarpic specimen. Fig. 2. Topotype, tetrasporophyte. Fig. 3. Young blade tip of sterile specimen, showing marginal meristem. Fig. 4. Thallus margin showing rhizoids. Fig. 5. Mature spermatangial sori. Fig. 6. Close-up of spermatangial sorus.

a marginal meristem (Fig. 3), later also by abundant intercalary cell divisions, and are monostromatic except in the immediate vicinity of reproductive structures, where they reach four cell layers in thickness. Perforations measure 200−300 μm in diameter and are commonly scattered over mature frond surfaces (Fig. 1) and not normally the result of shed reproductive sori. The vegetative cells are multi-



Figs 7−9. *Martensia martensii* (Schmitz *in* Schmitz *et* Hauptfleisch) Lin, Fredericq *et* Liao, comb. nov. (La Vista del Mar, Zamboanga, Philippines).
Fig. 7. View of carposporophyte after excision of pericarp (stained with aniline blue). Fig. 8. Cross-section through mature cystocarp showing enlarged nucleus in basal gonimoblast cell (arrow) and enlarged pit connections between basal gonimoblast cell and inner gonimoblasts (arrowheads) (stained with haematoxylin). Fig. 9. Tetrasporangial sori.

nucleate and abundantly linked by secondary pit-connections.

Reproductive morphology

Gametophytes are dioecious. Spermatangial sori are scattered over fertile male fronds and leave perforations 100−200 μm in diameter when the spermatia shed (Figs 5, 6).

Procarps were not observed. Young cystocarps are located along the thallus margins, whereas mature cystocarps are scattered across the blades, reaching 350−450 μm in diameter. Carposporangia mature sequentially and appear terminal on a candelabrum-like carposporophyte (Fig. 7). All gonimoblast cells remain uninucleate and distinct, with the basal gonimoblast cell containing an enlarged nucleus and not undergoing fusions with inner gonimoblast cells (Fig. 8). Primary pit-connections between gonimoblast cells broaden significantly (Fig. 8) but secondary pit-connections are lacking throughout the carposporophyte.

Tetrasporangial sori are minute, rounded to linear, reach 50−110 μm in diameter, and are scattered over the fertile blades (Fig. 9). Individual tetrasporangia measure 85−95 μm by 85−95 μm.

DNA sequence analysis

The *Rbc*L and partial LSU rDNA sequences were generated for 9 taxa in the Martensieae and 3 taxa in the Nitophylleae, the two tribes belonging to the emended subfamily Nitophylloideae of the Delesseriaceae. The outgroup consisted of the type species of both the subfamily Delesserioideae (*Delesseria sanguinea*) and subfamily Phycodryoideae (*Phycodrys rubens*) using the same total DNA vouchers.

The LSU rDNA alignment included 1712 sites. A large number of insertions/deletions (indels) were required in the data set to align the 5′ and 3′ ends of these sequences, and due to questionable homology of sites in these areas they were not included in analyses. Additionally, an internal portion within the sequence alignment was excluded so that the final data matrix included a total of 1253 sites of which 398 were parsimony informative (31.8%). The *rbc*L alignment consisted of 1412 sites and included 326 parsimony informative sites (23.1%).

Parsimony analyses obtained from multiple heuristic searches of the LSU rDNA and *rbc*L alignments are presented in two phylogenetic trees. Although distance and maximum likelihood trees are not presented, they were congruent with the parsimony trees. Only bootstrap values >50 are included.

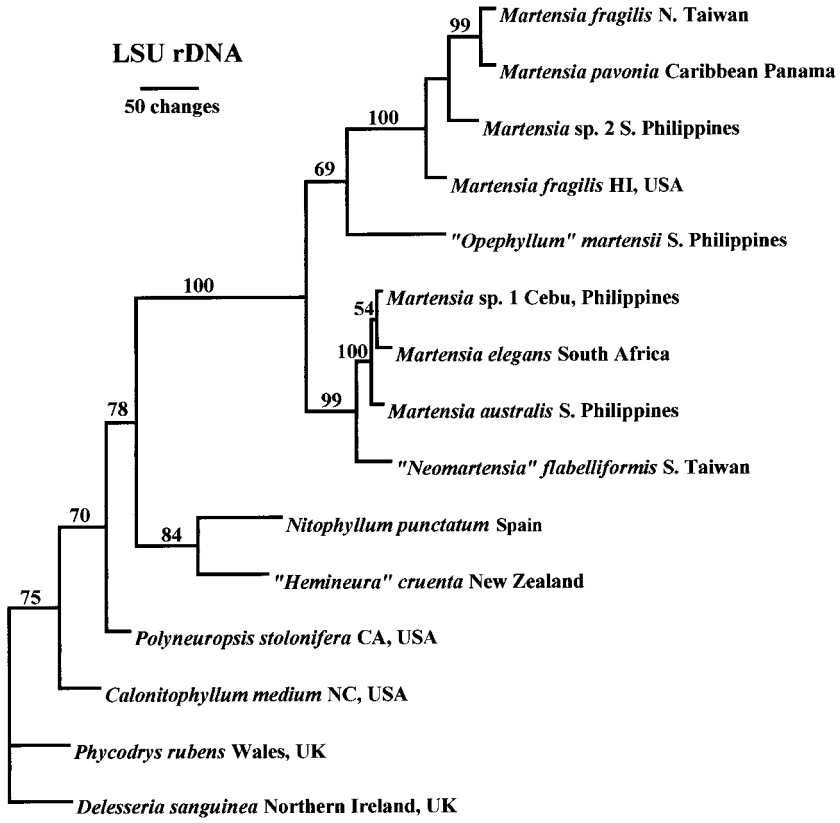


Fig. 10. One of the two most parsimonious trees inferred from LSU rDNA sequence analysis showing nested position of *Opephyllum* within the Martensieae. Tree length = 1083 steps, CI = 0.7498, RI = 0.7862, informative characters = 398 out of 1253 included sites. Bootstrap proportion values (1000 replicates, > 50%) are shown above nodes. Branch lengths are proportional to the amount of sequence change.

In both the LSU rDNA (Fig. 10) and *rbc*L (Fig. 11) analyses, the Martensieae comprised a strongly supported clade consisting of two well supported clusters of species placed in *Martensia. Opephyllum martensii* consistently was basal to a group containing both Indo-Pacific and Atlantic species of *Martensia*. The other group is Indo-Pacific and includes the type species, *M. elegans* from South Africa, and '*Neomartensia*' *flabelliformis*. The nested position of *Opephyllum* within *Martensia* was identical in the two analyses.

The Nitophylleae form a grade basal to the Martensieae. The type species of *Nitophyllum, N. punctatum,* clustered with a taxon from New Zealand belonging to *Valeriemaya* (Millar and Nelson personal communication) presently known as *Hemineura cruenta*.

Discussion and Conclusion

Lin *et al*. (2001) emended the subfamily Nitophylloideae to include the tribe Nitophylleae (*Nitophyllum*, *Polyneuropsis*, *Calonitophyllum*, *Valeriemaya,* and possibly *Papenfussia*) and the tribe Martensieae (*Martensia* including *Neomartensia*, and *Opephyllum)*, and erected a new subfamily, the Phycodryoideae, to include the remainder of taxa placed in the Nitophylloideae of Kylin (summarized in 1956).

The Martensieae typically have thalli without nerves or veins that are either perforated with small regular holes or form a marginal latticework. Cystocarps are distributed along the perforations or along the lattice with the ostioles emerging on one side, and tetrasporangia are organized in small round sori distributed over the thallus surface or within the lattice work (Millar 1990, Lin *et al*. 2001). The main features of *Opephyllum* are the monostromatic and membranous thallus, initial blade growth by a marginal meristem, lack of both microscopic veins and reticulate blade perforations, the presence of apparently programmed rounded perforations on older blades, scattered cystocarps in which cells of the carposporophytic filaments are linked by broadened primary pit-connections, lack of a post-fertilization fusion cell, and the fact that the basal gonimoblast cell remains small, is unfused to surrounding cells, and contains a single enlarged nucleus.

Opephyllum conforms in every particular that we have been able to observe save by its lack of reticulate meshworks to the defining features of *Martensia*, particularly in the development of the carposporophyte. Because procarps are lacking in our material, their precise makeup relative to superficially similar genera such as *Nitophyllum* and *Valeriemaya* cannot be determined. As young thalli of *Martensia* typically lack a network (personal observation), we view that in *Opephyllum martensii* the retention of these juvenile

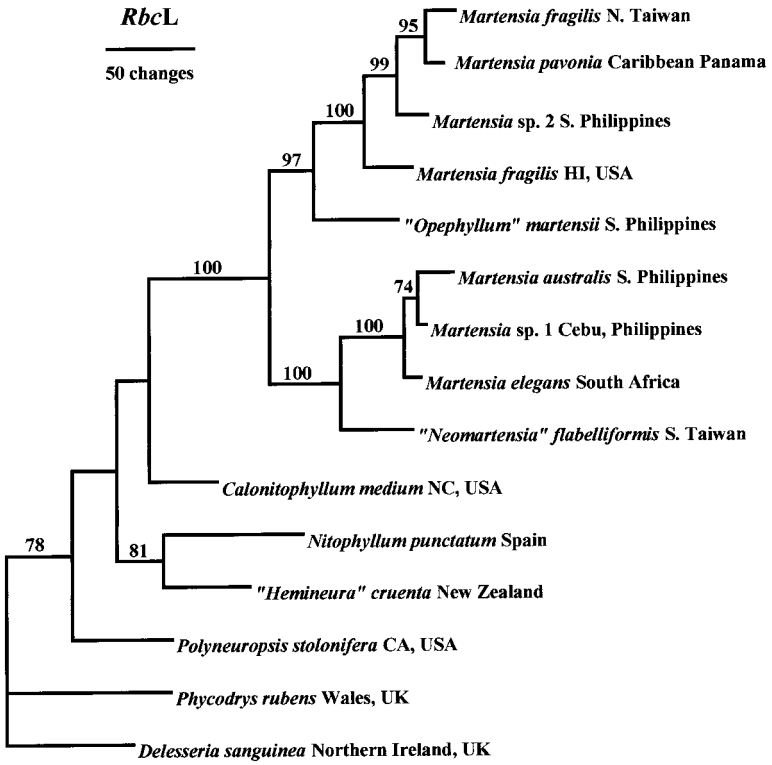


Fig. 11. Single-most parsimonious tree from *rbc*L sequence analysis showing nested position of *Opephyllum* within the Martensieae. Tree length = 953 steps, CI = 0.6275, RI = 0.6255, informative characters = 326 out of 1412 included sites. Bootstrap proportion values (1000 replicates, > 50%) are shown above nodes. Branch lengths are proportional to the amount of sequence change.

body features in the adult may be an example of paedomorphosis. Morphological considerations therefore, plus the indications reported herein of comparative molecular analyses of both chloroplast-encoded *rbc*L and the nuclear large-subunit ribosomal gene (LSU rDNA) which show *Opephyllum* to be nested within *Martensia,* lead us to propose the following transfer of the sole species of *Opephyllum* to *Martensia*, and to regard the former genus as a taxonomic synonym of the latter, confirming Papenfuss' speculation that both genera are congeneric. A revision of the genus *Martensia* is called for.

Martensia martensii (Schmitz *in* Schmitz *et* Hauptfleisch) Lin, Fredericq *et* Liao, comb. nov.

Basionym: *Opephyllum martensii* Schmitz *in* Schmitz *et* Hauptfleisch (1897. Delesseriaceae. *In*: A. Engler and K. Prantl, eds*, Die Natürlichen Pflanzenfamilien*…I. Teil, Abt. 2. Leipzig. p. 410).

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