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A new red algal species *Meristotheca dakarensis* (Solieriaceae, Gigartinales) from Senegal, western Africa, with comments on the relegation of *Meristiella* Cheny to synonymy with *Meristotheca* J. Agardh[1]

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Abstract — The marine red alga *Meristotheca dakarensis* Faye *et* Masuda, sp. nov. (Solieriaceae, Gigartinales) is described on the basis of material collected at Dakar, Senegal, western Africa and distinguished from other members of the genus by the following combination of morphological features: 1) irregularly dichotomously branched, thick, fleshy, tough, decumbent blades 10-30 cm in length and 500-1200 µm in thickness, with segments 1-4 cm in width; 2) tetrasporangial initials cut off basally from their parental cells; 3) dioecious gametophytes; 4) (2-)3(-4)-celled carpogonial branches, the basal cell of which rarely bears a single lateral cell; 5) the presence of an auxiliary-cell complex; and 6) the production of numerous protuberant cystocarps on the dorsal surfaces (less frequently on the ventral surfaces and margins) of the mid to distal portions of the blades. Molecular-phylogenetic analyses based on *rbc*L gene sequences indicate that *Meristotheca dakarensis* is contained within a monophyletic clade that includes species currently placed in either *Meristiella* and *Meristotheca*, the clade becoming paraphyletic with the recognition of both genera. Pairwise distances between *M. dakarensis* and other species of the genus complex are large, ranging from 44 bp to 64 bp (4.3% to 5.1%). Anatomical features previously used to distinguish *Meristiella* Cheny from *Meristotheca* J. Agardh (the absence of carpogonial nemathecia; the presence of an auxiliary-cell complex; cystocarps that bear conspicuous spines) are shown to be inconsistently displayed among species of the clade, and it is proposed as a result of both morphological and molecular data that *Meristiella* be subsumed in *Meristotheca*, the necessary new combinations being made as a consequence.

***Meristotheca dakarensis* / *Meristotheca echinocarpa* / *Meristotheca gelidium* / *Meristotheca schrammii* / molecular phylogeny / morphology / *rbc*L / Rhodophyta / Solieriaceae / taxonomy**

1. This paper is dedicated to Professor Isabella A. Abbott to honour her 85th birthday.

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Résumé — Une nouvelle espèce d'algue rouge, *Meristotheca dakarensis* (Solieriaceae, Gigartinales), du Sénégal, Afrique occidentale, avec commentaires sur la mise en synonymie de *Meristiella* Cheny avec *Meristotheca* J. Agardh. L'algue rouge marine *Meristotheca dakarensis* Faye *et* Masuda, sp. nov. (Solieriaceae, Gigartinales) est décrite à partir de matériel récolté à Dakar, Sénégal (Afrique occidentale). Elle se distingue des autres membres du genre par la combinaison suivante de caractères morphologiques : 1) ramification irrégulièrement dichotomique, épaisse, molle, résistante, à lames décombantes de 10-30 cm de longueur et de 500-1200 µm d'épaisseur, avec des segments de 1-4 cm de largeur ; 2) initiales des tétrasporocystes se découpant à la base de leurs cellules-mères ; 3) gamétophytes dioïques; 4) rameaux carpogoniaux de (2-)3(-4) cellules, dont la cellule basale porte rarement une seule cellule latérale ; 5) la présence d'un complexe cellule auxiliaire + cellules végétatives adjacentes ; et 6) production de nombreux cystocarpes protubérants sur les surfaces dorsales (moins fréquemment sur les surfaces ventrales et les marges) au milieu des parties distales des lames. Les analyses de phylogénie moléculaire basées sur les séquences du gène *rbc*L indiquent que *Meristotheca dakarensis* est contenu dans un clade monophylétique qui englobe des espèces couramment placées à la fois dans *Meristiella* et *Meristotheca* ; le clade devenant paraphylétique avec la reconnaissance des deux genres. Les distances par comparaison des séquences deux à deux entre *M. dakarensis* et les autres espèces du complexe générique sont grandes, atteignant de 44 bp à 64 bp (4,3 % à 5,1 %). Les caractères anatomiques précédemment utilisés pour distinguer *Meristiella* Cheney de *Meristotheca* J. Agardh (l'absence de némathécie carpogoniale, la présence d'un complexe cellule auxiliaire + cellules végétatives adjacentes, les cystocarpes portant des épines bien visibles) se montrent répartis de façon non cohérente parmi les espèces du clade ; il est donc proposé, compte tenu des résultats des données morphologiques et moléculaires, d'inclure *Meristiella* dans *Meristotheca*, les nouvelles combinaisons rendues nécessaires étant faites en conséquence. (Traduit par la Rédaction)

***Meristotheca dakarensis* / *Meristotheca echinocarpa* / *Meristotheca gelidium* / *Meristotheca schrammii* / morphologie / phylogénie moléculaire / *rbc*L / Rhodophyta / Solieriaceae / taxinomie**

INTRODUCTION

The red algal genus *Meristotheca* J. Agardh (1872) (Solieriaceae, Gigartinales) was erected to accommodate two species, *M. papulosa* (Montagne) J. Agardh, which was originally described as *Kallymenia papulosa* Montagne (1850, as *Callymenia*), and *M. duchassaingii* J. Agardh. Since then, a total of twelve species has been assigned to the genus, although four have been removed to other genera: *M. decumbens* Grunow (in Piccone, 1884) to *Rhabdonia*, as *R. decumbens* (Grunow) Grunow (in Askenasy, 1888); *M. duchassaingii* to *Halymenia*, as *H. duchassaingii* (J. Agardh) Kylin (1932); *M. floridana* Kylin (1932) to *Agardhiella*, as *A. floridana* (Kylin) Guimarães *et* Oliveira (1996); and *M. natalensis* J. Agardh (1876) to *Cryptonemia*, as *C. natalensis* (J. Agardh) Chiang (1970). Neto *et al.* (2002) recently reported an alga, which they identified as *M. decumbens,* from the Azores. As their specimens bear "irregularly cruciate tetraspores", the alga is likely to be unrelated to the Solieriaceae, in which members always produce zonate tetrasporangia. The following three species have been reduced to synonymy with other species: *M. gigartinoides* Joly *et* Ugadim (in Joly *et al*., 1965) with *Agardhiella floridana*; *M. japonica* Kylin (1932) with *M. papulosa* (Okamura, 1936); and *M. tasmanica* J. Agardh (1876) with *Austrophyllis harveyana* (J. Agardh) Womersley *et* Norris (Womersley, 1994). The genus thus currently includes the fol-

lowing five species, which are distributed in tropical and warm-temperate regions in the world: *M. coacta* Okamura (1930), *M. fergusonii* Grunow *ex* Mazza (1920), *M. papulosa*, *M. procumbens* Gabrielson *et* Kraft (1984) and *M. tobagensis* Taylor (1962).

A further species of *Meristotheca, M. senegalensis* J. Feldmann, has been listed in the literature pertaining to the marine algal flora of Senegal, western Africa (Sourie, 1954; Bodard & Mollion, 1974; Harper & Garbary, 1997). In a study of some potential sources of iota carrageenans from Senegal, Fostier (1989) stated as follows: "When we decided to study this alga [*M. senegalensis*], we were based upon the previous works of Sourie (1954) and Bodard & Mollion (1974) and, all of them attributed the alga to J. Feldmann. At our request, a further search of the holdings at the Herbarium of the Muséum National d'Histoire Naturelle, Paris, was kindly undertaken by F. Ardré, who reports that although it was J. Feldmann who introduced the nomen nudum *Meristotheca senegalensis*, the current whereabouts of voucher material on which he based the name is unknown". Recently, Watt *et al.* (2003) also reported a similar unsuccessful search for authentic Feldmann material of *M. senegalensis*. Based on gross morphological features, Fostier (1989) further indicated that the possibility exists that *M. senegalensis* may be equivalent to material from Dakar identified as *Sarcodia ceylanica* Harvey by Dangeard (1952), but in the absence of authentic material of both *Meristotheca senegalensis* and *Sarcodia ceylanica* we agree with Fostier's (1989) decision to provisionally identify *Meristotheca*-like plants from Senegal with Feldmann's manuscript name rather than *Sarcodia ceylanica*. In the present study, detailed morphological observations, in combination with *rbc*L gene-sequence analyses, are reported in order to clarify the precise taxonomic status of the Senegalese alga currently passing under the name *Meristotheca senegalensis* or possibly *Sarcodia ceylanica.*

MATERIALS AND METHODS

Material examined

Material examined was collected at Ngor Island, located in the Cap-Vert Peninsula, Dakar, Senegal, and fixed in 10% formalin/seawater or dried on herbarium papers. Voucher herbarium specimens from the following three collections are deposited in the Herbarium of the Graduate School of Science, Hokkaido University, Sapporo: 1) 31 July 2002, *leg*. E. J. Faye, tetrasporangial (SAP 096653, 096654), spermatangial (SAP 096655) and cystocarpic (SAP 096656); 2) 18 January 2003, *leg*. G. Dioh Faye, vegetative (SAP 096657-096661); and 3) 10 May 2003, *leg*. G. Dioh Faye, vegetative (SAP 096662), tetrasporangial (SAP 096663) and cystocarpic (SAP 096664-096666).

Anatomical observations

Anatomical observations were made on 10% formalin/seawater-preserved material or on rehydrated herbarium specimens. Sections were made on a freezing microtome at 40 μm or by hand (using a razor blade and pith stick), stained with 0.5% (w/v) Cotton blue solution [lactic acid/phenol/glycerol/water (1:1:1:1) (v/v)], and mounted in 50% glycerol/seawater or 30% Karo on microscope slides.

*rbc*L analyses

Total DNA was extracted from three samples of *Meristotheca dakarensis* [GenBank accession numbers (voucher specimen numbers in SAP): AB159224 (SAP 096653), AB159225 (SAP 096656) and AB159226 (SAP 096658)] using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) following the protocol of the manufacturer. The total DNA was used as template for the polymerase chain reaction (PCR). The following two pairs of primers were used for amplification of the *rbc*L gene: F-118 (5'-TCCACAACCAGGTGTGGATCC-3) — R-831 (5'-TCATTTTTACGAGCCCAAA-3'), and F-737 (5'-ATGTATGAGAGAGCT-GAATT-3') — R-1425 (5'-TTATACGTTAGCTGTTGGAG-3'). They were newly designed based on *rbc*L sequences of selected solieriacean algae downloaded from GenBank. The conditions of amplification consisted of an initial denaturation step of 93°C for 1 min, followed by 35 cycles of 30 sec, denaturation period at 94°C, 30 sec primers annealing at 50°C and 45 sec extension at 72°C, terminated by a final hold at 4°C. These temperature-cycling reactions were run on a Perkin-Elmer GenAmp PCR system 9600 or 2400 (Applied Biosystems, CA, USA). The presence of the PCR-amplified products was verified by agarose gel electrophoresis, followed by staining with Ethidium Bromide. Prior to cycle-sequencing, PCR products were purified by PEG precipitation and directly sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit ver. 1.1 (Applied Biosystems, CA, USA) according to the manufacturers' protocol. Cycle-sequencing reactions were run on a Perkin-Elmer GenAmp PCR system 9600 or 2400 and consisted of an initial step of 96°C for 10 sec, followed by 25 cycles (96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min) and a final hold at 4°C. Both forward and reverse strands were sequenced using a DNA autosequencer (ABI PRISM, 310 Genetic Analyzer, Applied Biosystems, CA, USA).

The *rbc*L sequences were aligned manually because no insertion–deletion mutations were detected. Sequences of the following 27 species of the Solieriaceae were downloaded from GenBank and included in the alignment: *Agardhiella ramosissima* (Harvey) Kylin (AF099680), *A. subulata* (C. Agardh) Kraft *et* Wynne (U04176), an unidentified species of *Agardhiella* (AF099681), *Anatheca montagnei* Schmitz (AB122014), *Betaphycus philippinensis* Doty (AF099684), *B. speciosum* (Sonder) Doty (AF099685), *Eucheuma arnoldii* Weber-van Bosse (AF099690), *E. denticulatum* (Burman) Collins *et* Hervey (U04177), *E. isiforme* (C. Agardh) J. Agardh (AF099691), *E. serra* (J. Agardh) J. Agardh (AF099692), *Kappaphycus alvarezii* (Doty) Doty *ex* Silva (AF099694), *K. cottonii* (Weber-van Bosse) Doty *ex* Silva (AF099695), *K. striatum* (Schmitz) Doty *ex* Silva (AF099696), an unidentified species of *Kappaphycus* (AF481500), *Meristiella gelidium* (J. Agardh) Cheney *et* Gabrielson (AF099697, AF099698), an unidentified species of "*Meristiella*" (AF099699), *Meristotheca papulosa* (Montagne) J. Agardh (AF099700), *M. procumbens* Gabrielson *et* Kraft (AF099701, AF099702), an unidentified species of *Meristotheca* (AF099703), *Sarcodiotheca furcata* (Setchell *et* Gardner) Kylin (AF099706), *S. gaudichaudii* (Montagne) Gabrielson (U04184. AF099707), *Sarconema filiforme* (Sonder) Kylin (AF099708), *Solieria chordalis* (C. Agardh) J. Agardh (AF099709), *S. filiformis* (Kützing) Gabrielson (U04185), *S. pacifica* (Yamada) Yoshida (AF099710), *S. robusta* (Greville) Kylin (AF099711) and an unidentified species of *Solieria* (AF099712). Two species of the Caulacanthaceae, *Caulacanthus ustulatus* (Turner) Kützing (AF099687) and *Heringia mirabilis* (C. Agardh) J. Agardh (U21601) and a species of Areschougiaceae, *Areschougia ligulata* Harvey *ex* J. Agardh (AF099683), were used as outgroups for the phylogenetic analysis. The alignment is available from the second author upon request.

Maximum parsimony (MP) and maximum likelihood (ML) methods were used to construct phylogenetic trees. Parsimony analysis was performed with PAUP 4.0 b10 (Swofford, 2002), with all sites treated as unordered and equally weighted. Heuristic search option with random addition of sequences (100 replicates) and tree-bisection-reconnection branch swapping algorithm (TBR) was used for tree searching. Bootstrap analysis based on 2000 re-samplings of the data set (Felsenstein, 1985) was calculated (10 random additions, TBR, Full heuristic search option) to evaluate statistical reliability.

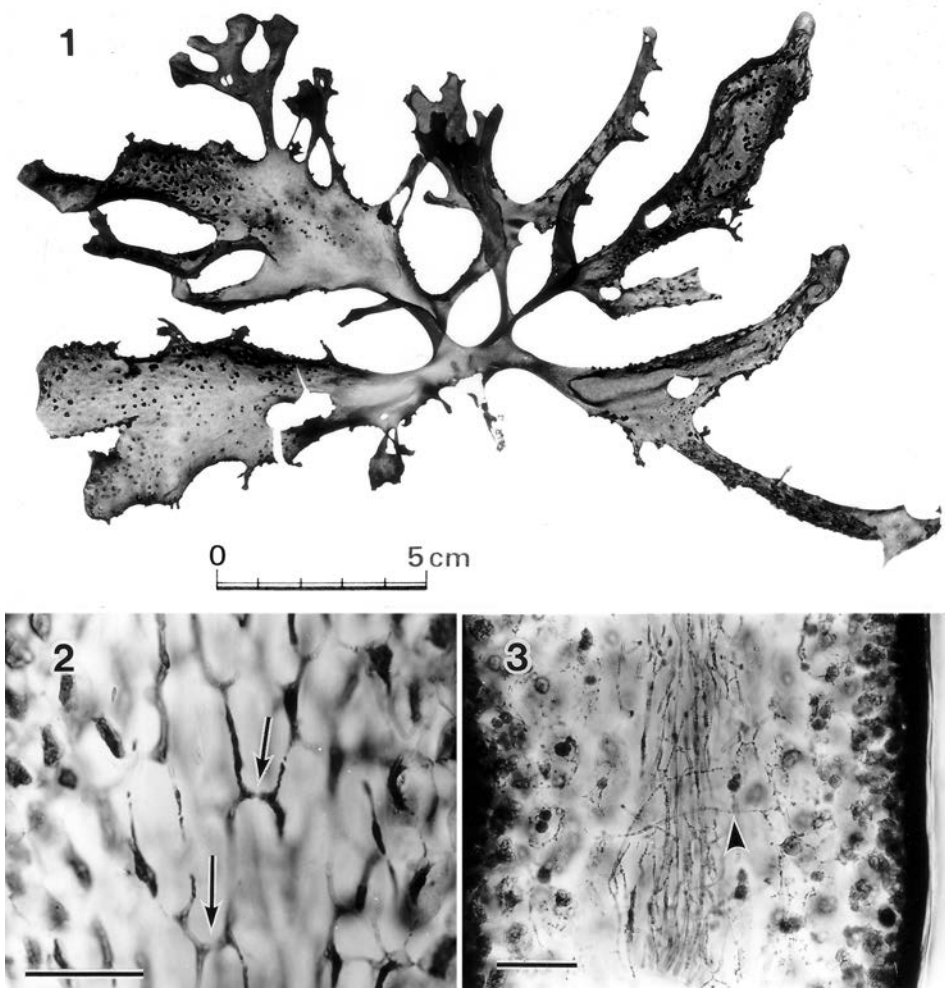
The maximum likelihood analysis was also implemented with PAUP 4.0b10. The ML parameters were estimated using the ML ratio test. The program MODELTEST version 3.06 (Posada & Crandall, 1998) was used to find the model of sequence evolution that best fitted each data set by a hierarchical likelihood ratio test ($\alpha = 0.01$). When the best sequence evolution model was determined, ML tree search was performed using the estimated parameters with the following options: starting tree option = obtained by neighbour joining, and branch swapping algorithm = TBR. Bootstrap analysis based on 100 re-samplings of the data set (Felsenstein, 1985) was calculated (TBR, full heuristic search option) to evaluate statistical reliability.

RESULTS

Habitat and vegetative structures

Thalli grow as unaggregated individuals on bedrock in the upper subtidal zone between 0 and 6m. Each thallus consists of one or a few decumbent blades (Fig. 1) that arise from a single discoid primary holdfast and reach 10-30 cm in length when reproductive. Blades are 1000-1200 µm thick proximally, thinning gradually distally to thicknesses of 500-600 µm one mm proximal to the apices. Lower portions of blades are attached to the substratum by secondary attachment discs, in addition to the primary one, that develop from the margins and ventral surfaces closely adjacent to the margins. Reproductive blades are deeply rose-red or reddish-brown in colour and fleshy but tough in consistency. They branch irregularly dichotomously four to twelve times and produce segments 1-4 cm in width. Margins are smooth throughout or proliferous in lower to mid portions of blades, the proliferations either small, simple and laciniate (up to 2 mm in length), or large, foliose, and dichotomously divided two to four times (up to 7 cm in length). Prostrate portions may form anastomoses where they overlap. Surfaces of blades are often maculate regardless of their reproductive state.

Blades are multiaxial and internally composed of a thin, filamentous medulla and a thick, pseudoparenchymatous cortex. The medulla occupies about one-third to one-eighth of the blade thickness and consists of primary axial filaments and adventitious filaments. Axial filaments 10-20 µm in diameter run parallel to the longitudinal axes of the blades (Figs 2, 3) and bear a single periaxial cell from each segment, the periaxial cells subsequently producing lateral filaments that constitute the cortex. Secondary pit-connections between adjacent medullary cells are frequent (Fig. 2), although interconnecting filaments or cells linking adjacent medullary filaments are absent. Adventitious medullary filaments, which are



Figs 1-3. *Meristotheca dakarensis*. Fig. 1. Holotype specimen (cystocarpic, SAP 096664). Fig. 2. Longitudinal section (LS) of a young blade (uppermost portion) stained with Cotton blue: arrows indicating secondary pit-connections between adjacent medullary cells. Fig. 3. LS of a mature female blade (upper portion) stained with Cotton blue: arrowhead indicates an adventitious medullary filament traversing the blade and connecting two inner cortical cells. Scale bar = 50 μm in Figs 2, 3.

formed from axial filaments and grow towards inner cortical cells to secondarily connect with those cells, are abundant. In lower portions of mature blades such adventitious filaments actually enter the cortex and form secondary connections to cells as few as four layers in from the blade surfaces. Adventitious filaments also arise on inner cortical cells (although in lesser numbers than those from axial filaments), traverse the blade and connect with inner cortical cells of the opposite side (Fig. 3).

The cortex is composed of four or five inner layers of large (40-250 μm in diameter), unpigmented or lightly pigmented, ellipsoidal or rounded cells that are laterally linked by secondary pit-connections, these subtending three to five outer layers of much smaller (5-15 μm in diameter), deeply pigmented ellipsoidal cells that are also secondarily pit-connected with the exception of cells of the outermost one or two layers.

Reproductive structures

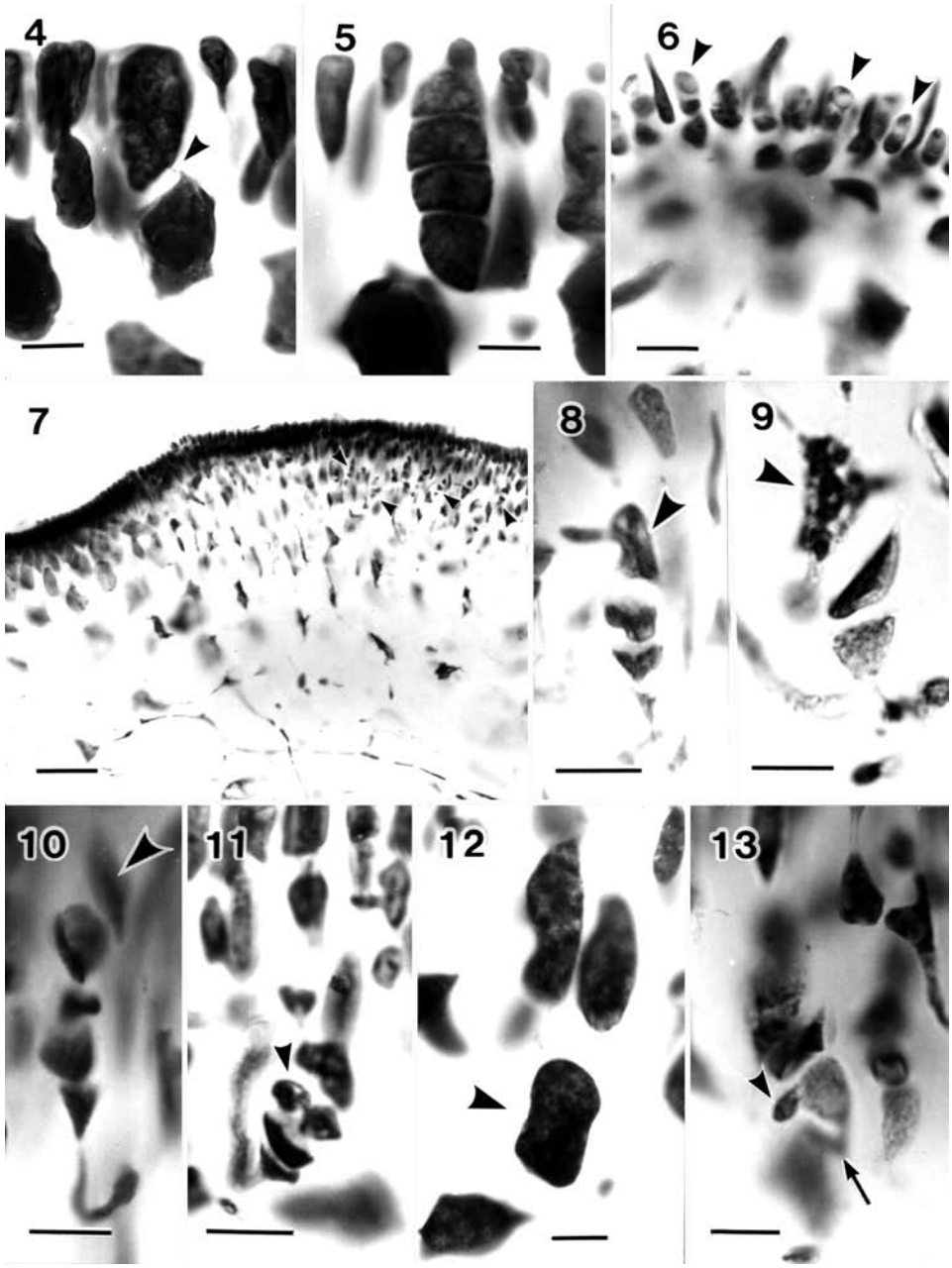
Tetrasporangia are formed in scattered, unswollen sori over entire tetrasporophytic blades from one or two cm distal to the bases, although the sori are more abundant on dorsal than on ventral sides. Tetrasporangial initials are pit-connected basally to parent cells two or three layers in from the surface (Fig. 4), divide zonately (Fig. 5) and measure 38-45 μm in length by 15-20 μm in diameter at maturity.

Spermatangia are formed over both surfaces of male gametophytes except at the bases, one or two spermatangia being cut off from surface spermatangial parent cells (Fig. 6). Mature spermatangia are ellipsoidal, 4-5 μm long by 3-4 μm in diameter. Elongated sterile surface cortical cells are frequently intercalated like paraphyses among the spermatangial parental cells before (Fig. 6) and after release of spermatia.

Carpogonial branches and auxiliary cells are present in distal, slightly raised portions (Fig. 7) on female gametophytes. The carpogonial branch is produced laterally or basally from an intercalary supporting cell situated in the third to ninth cortical layers in from the surface. At first directed inwardly, at maturity it bears a trichogyne that is reflexed towards the blade surface. Carpogonial branches are usually three-celled, although occasionally they are two- or four-celled (Figs 8-10). Carpogonia are conical, 5-10 μm in diameter, and narrow distally to a trichogyne that is up to 200 μm in length and 4 μm in diameter. Hypogynous cells are barrel-shaped, 4-8 μm in length by 6-10 μm in diameter, whereas basal cells (and also the suprabasal cells when carpogonial branches are four-celled) are also barrel-shaped, 5-10 μm in length by 8-10 μm in diameter. A single-celled sterile lateral is rarely formed on the basal cell (Fig. 11).

Auxiliary cells contain a single, large and darkly-staining nucleus (Fig. 12) as opposed to multinucleate surrounding cortical cells. Prior to diploidization, the auxiliary cell is ellipsoidal in shape, 25-32 μm in length by 15-18 μm in diameter, and together with associated vegetative cells stains more deeply to form the auxiliary-cell complex (Fig. 12).

The fertilized carpogonium produces a lateral protuberance (Fig. 13) growing into an unbranched, non-septate connecting filament (Fig. 14) that fuses laterally with an auxiliary cell situated in the fourth to ninth cortical layers in from the surface. Following diploidization, inner-cortical cells surrounding the auxiliary cell divide to form nutritive-cell clusters (Fig. 15), after which the auxiliary cell cuts off a single gonimoblast initial laterally (Fig. 16), the initial in turn further dividing to produce a dense cluster of darkly-staining gonimoblast cells (Figs 17, 18). The nutritive-cell clusters intermingle with radially stretching cells of the surrounding cortex to produce a distinct envelope of sterile tissue. At the same time, surface cells that lie directly above the diploidized auxiliary cell become meristematic and form an elevated, ostiolate pericarp that partially surrounds the developing carposporophyte. Tubular gonimoblast cells that fuse with or connect with cells of the enveloping tissue are present in young (Fig. 18) to mature cystocarps.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Further development of the carposporophyte results in a centrally placentate cystocarp (Fig. 19) with peripheral, unbranched (occasionally sparingly branched) chains of up to seven ellipsoidal or obovoid carposporangia 20-26 µm by 14-20 µm (Fig. 20).

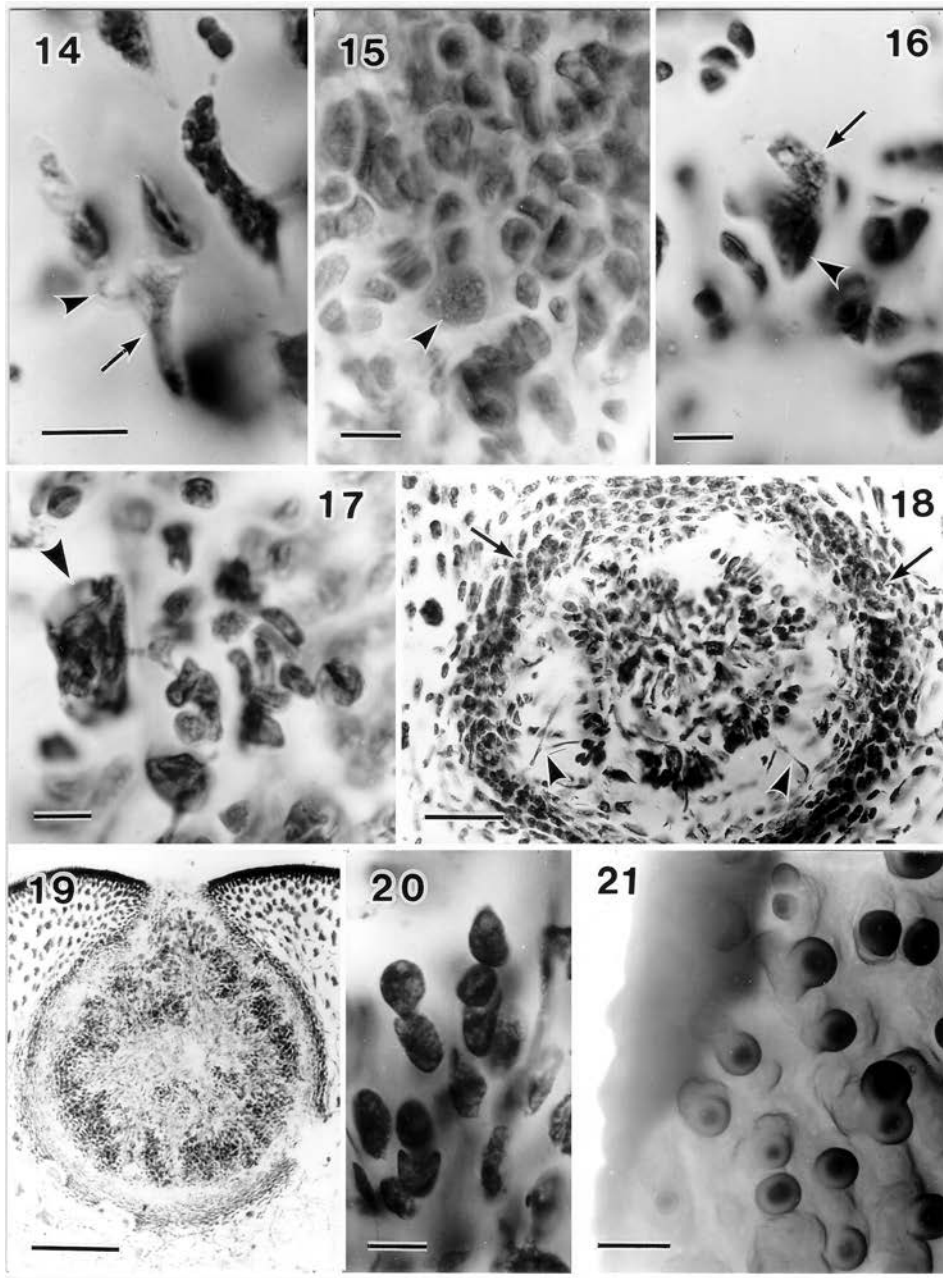
Cystocarps (Fig. 21) are 750-850 µm in height by 700-800 µm in diameter (including an enveloping tissue 50-80 µm thick except an ostiolate pericarp) and are numerous but mostly confined to mid to distal dorsal blade surfaces, although a few may be present on ventral sides and margins (Fig. 1). Closely adjacent carposporophytes can partially fuse into a single protuberance 0.5-2.0 mm in height by 1.5-2.8 mm in diameter that is provided with plural ostioles corresponding to the number of carposporophytes.

*rbc*L analysis

Three samples from Senegal were sequenced, but because sequences were incomplete at the 5' and 3' ends, 1295 base pairs (bp) corresponding to positions 110-1404 of the *rbc*L sequence of *Chondrus crispus* Stackhouse (U02984) were used for the alignment. Sequences of all three samples were identical.

Phylogenetic trees obtained from MP and ML analyses are presented in Figs 22 and 23. Identical sequences of our alga were excluded from the alignment for the phylogenetic analyses. In the MP analysis, the single, most-parsimonious tree (1187 steps, CI = 0.496, RI = 0.563, RC = 0.279) was found and is presented in Fig. 22. For the ML method, likelihood settings from the best-fit model (GTR + I + G) were selected by a hierarchical likehood ratio test in the program MODELTEST version 3.06: assumed nucleotide frequencies A = 0.3000, C = 0.1373, G = 0.2036, and T = 0.3591; substitution-rate matrix with AC = 1.1638, AG = 7.5514, AT = 2.08470, CG = 1.4616, CT = 16.4335, and GT = 1; proportion of invariable sites = 0.3856; gamma distribution with shape parameter = 0.5797. Based on these settings, the heuristic search was performed with the TBR branch swapping option (-ln L = 7669.07582) after 13147 rearrangements (Fig. 23). Topologies of the MP and ML trees were almost congruent, except for the clades that were supported with low bootstrap values. Our alga in question forms a monophyletic clade with species of *Meristiella* and *Meristotheca* (with relatively strong bootstrap support). Pairwise distances between the alga under study and other species of this clade range from 44 bp to 64 bp (4.3% to 5.1%).

Figs 4-13. *Meristotheca dakarensis*. Transverse sections (TS) or longitudinal sections (LS) stained with Cotton blue. Fig. 4. Young tetrasporangium that is basally pit-connected (arrowhead) to its parental cell (TS). Fig. 5. Zonately divided tetrasporangium (TS). Fig. 6. Spermatangia (arrowheads) produced from the outermost cortical cells (TS). Fig. 7. Slightly raised (nemathecium-like) portion containing many carpogonial branches (arrowheads) (LS). Fig. 8. Three-celled carpogonial branch: arrowhead indicates the supporting cell (LS). Fig. 9. Two-celled carpogonial branch: arrowhead indicates the supporting cell (LS). Fig. 10. Four-celled carpogonial branch: arrowhead indicates the supporting cell (LS). Fig. 11. Three-celled carpogonial branch having a sterile lateral (arrowhead) on the basal cell (LS). Fig. 12. Auxiliary cell complex: arrowhead indicates the auxiliary cell. Fig. 13. Mucronate initial of a connecting filament (arrowhead) developing from a carpogonium: arrow indicating a trichogyne. All scale bars = 10 µm.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DISCUSSION

The inwardly directed carpogonial branches, reflexed trichogynes, and connecting filaments that arise directly from fertilized carpogonia in the Senegalese alga are consistent features of solieriacean genera, including *Meristotheca* (Gabrielson & Kraft, 1984). Although features of the Senegal species such as the absence of interconnecting filaments, carpogonial branches and auxiliary cells formed in slightly raised portions, the occasional presence of a sterile cell on the basal cell of the carpogonial branch, a single connecting filament from each fertilized carpogonium, protuberant cystocarps (each consisting a centrally placentate carposporophyte, inner enveloping tissue and surrouding ostiolate pericarp), best agree with *Meristotheca* among solieriacean genera, they also largely fit the criteria for inclusion in *Meristiella*. The genus *Meristiella* Cheney was originally distinguished from *Meristotheca* by Gabrielson & Cheney (1987) on the basis of the following differences: 1) carpogonial branches and auxiliary cells are formed in slightly raised portions (as nemathecia *sensu* Gabrielson & Cheney, 1987) on the blade in *Meristotheca* but in non-raised portions in *Meristiella*; 2) an auxiliary-cell complex is absent in *Meristotheca* but present in *Meristiella*; and 3) cystocarps are smooth-walled in *Meristotheca* but bear conspicuous spines in *Meristiella*. However, based upon molecular-phylogenetic analyses using *rbc*L sequences that do not support their separation, Fredericq *et al*. (1999) suggest that the two genera should be merged as two of the just-mentioned morphological characters are difficult to confirm (they did not comment on the presence or absence of spines on a cystocarp). Our *rbc*L trees strongly support the suggestion of Fredericq *et al.* (1999) and highlight the difficulty in determining what are basically the statuses of continuously varying features in this suite of algae, such as the relative prominence or indistinctness of auxiliary-cell complexes and degree of "nemathecial" raising.

On the basis of the report of Fredericq *et al*. (1999) and the results that we report now from our own study, we recommend that the genus *Meristiella* Cheney (in Gabrielson & Cheney, 1987) be formally reduced to synonymy with *Meristotheca* J. Agardh. Consequent binomials and authorities for former species of *Meristiella*, including the recently described Chinese M. *florigia* Kuang *et* Xia (2003), are given below (Tab. 1).

The three "*Meristiella*" species shown in Table 1 differ from the Senegalese alga in the presence of spinose cystocarps, which we consider to have

Figs 14-21. *Meristotheca dakarensis*. Longitudinal sections (LS) stained with Cotton blue except for Fig. 21. Fig. 14. Connecting filament (arrow) developing from a carpogonium: arrowhead indicating a trichogyne. Fig. 15. Nutritive cell clusters formed from vegetative cells around the diploidized auxiliary cell (arrowhead). Fig. 16. Gonimoblast initial (arrowhead) from a diploidized auxiliary cell: arrow indicating a connecting filament. Fig. 17. Early lateral development of the gonimoblast from the auxiliary cell (arrowhead). Fig. 18. Early stage of gonimoblast development in which the carposporophyte is surrounded by enveloping filaments (arrows): arrowheads indicate tubular gonimoblast cells that contact with cells of the enveloping filaments. Fig. 19. Placentate cystocarp with an ostiole. Fig. 20. Branched chain of carposporangia. Fig. 21. Dorsal view of the holotype specimen (before press) having numerous cystocarpic protuberances. Scale bar = 10 μm in Figs 14-17, 20; scale bar = 50 μm in Fig. 18; scale bar = 200 μm in Fig. 19; scale bar = 2 mm in Fig. 21.

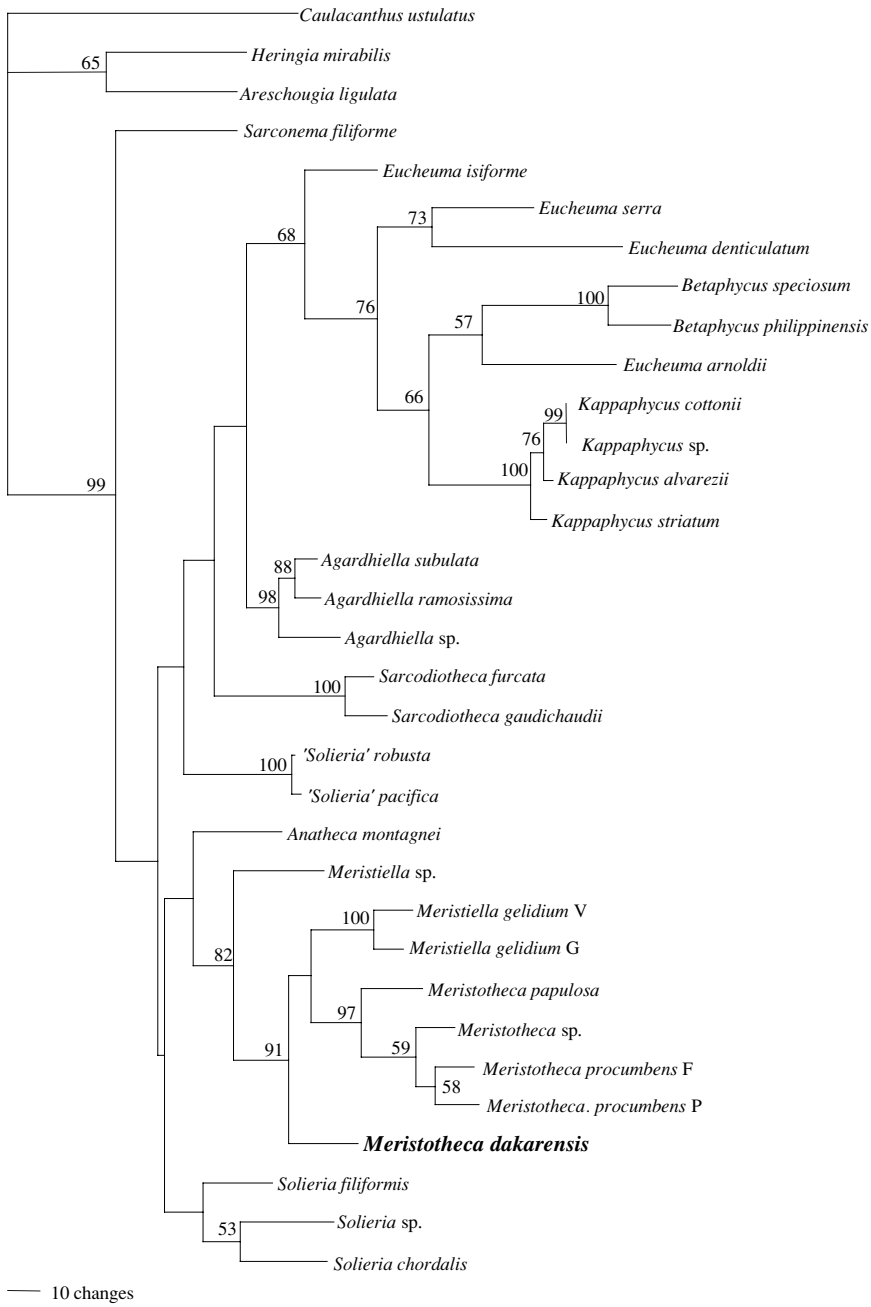

Fig. 22. Most parsimonious tree (1187 steps, CI = 0.496, RI = 0.563, RC = 0.279) of the Solieriaceae inferred from partial *rbc*L gene sequences (1295 bp). *Caulacanthus ustulatus*, *Heringia mirabilis* and *Areschougia ligulata* were used as outgroups. All sites were treated as unordered and equally weighted; only values above 50% bootstrap support (2000 replicates, full heuristic search with TBR method) are shown.

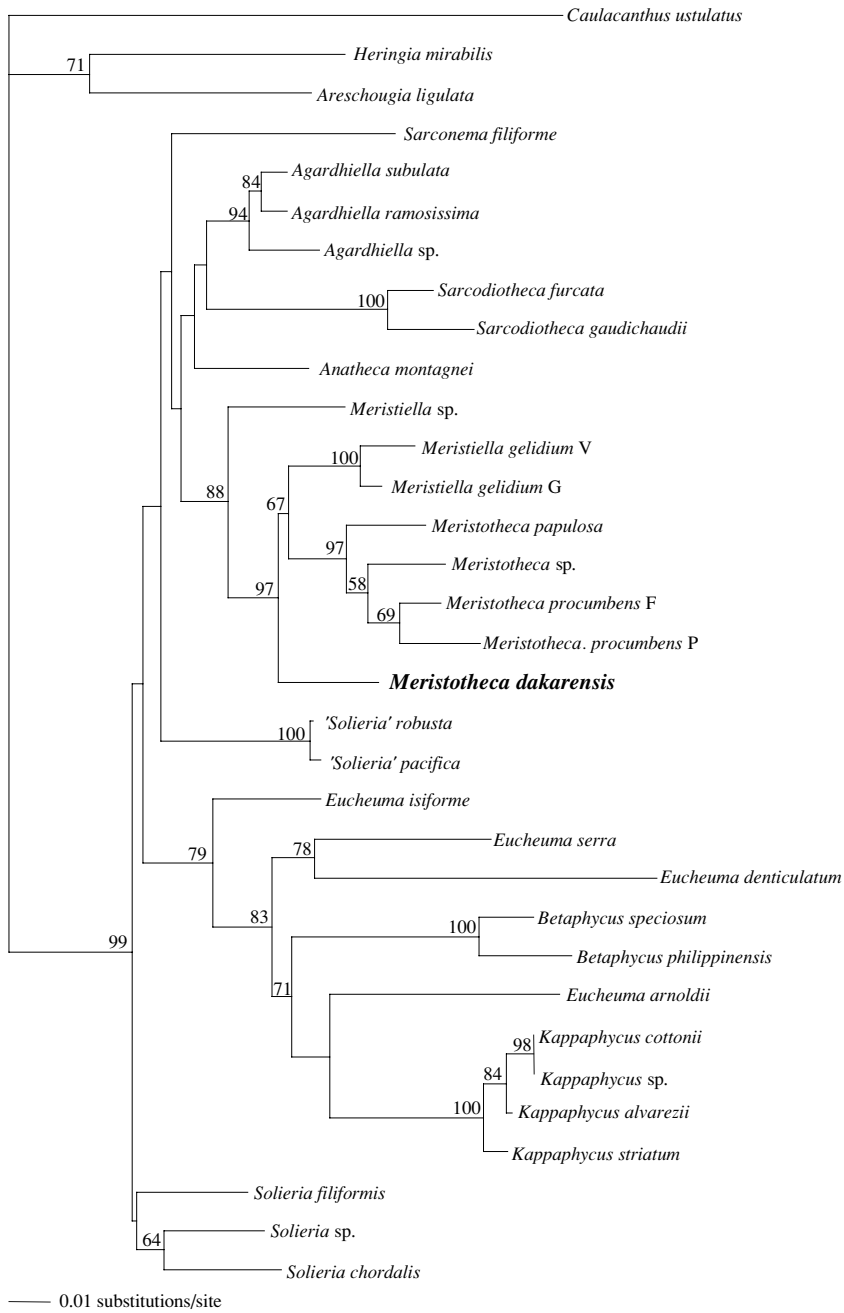

Fig. 23. Phylogenetic tree of the Solieriaceae inferred from ML analysis of partial *rbc*L gene sequences (1295 bp). *Caulacanthus ustulatus*, *Heringia mirabilis* and *Areschougia ligulata* were used as outgroups. Only values above 50% bootstrap support (100 replicates, full heuristic search with TBR method) are shown.

Table 1. Formal proposals of transfer of *Meristiella* species[1] to *Meristotheca*

1 ***Meristotheca echinocarpa* (Areschoug) Faye *et* Masuda, comb. nov.**
Basionym: *Eucheuma echinocarpum* Areschoug 1854: 349
Homotypic synonym: *Meristiella echinocarpa* (Areschoug) Cheney *et* Gabrielson *in* Gabrielson *et* Cheney 1987: 483

2 ***Meristotheca gelidium* (J. Agardh) Faye *et* Masuda, comb. nov.**
Basionym: *Sphaerococcus gelidium* J. Agardh 1841: 17
Homotypic synonyms: *Gigartina gelidium* (J. Agardh) Endlicher 1843: 42; *Eucheuma gelidium* (J. Agardh) J. Agardh 1847:16; *Meristiella gelidium* (J. Agardh) Cheney *et* Gabrielson *in* Gabrielson *et* Cheney 1987: 483

3 ***Meristotheca schrammii* (P. Crouan *et* H. Crouan) Faye *et* Masuda, comb. nov.**
Basionym: *Mychodea schrammii* P. Crouan *et* H. Crouan in Schramm *et* Mazé 1865: 10
Homotypic synonym: *Meristiella schrammii* (P. Crouan *et* H. Crouan) Cheney *et* Gabrielson *in* Gabrielson *et* Cheney 1987: 483

1. *Meristiella florigia*, described from China (Kuang & Xia, 2003) was excluded from this table because its generic status cannot be determined by their information (see Text).

taxonomic significance at the species rather than the genus level. In addition, thalli of these species all differ considerably (Gabrielson & Cheney, 1987) in blade orientation (erect as opposed to prostrate or decumbent) and branching (subopposite to alternate rather than subdichotomous) from *M. dakarensis*. Although details of diploidization processes, early gonimoblast development and tetrasporangia are not known for *Meristiella florigia,* that species clearly differs from *Meristotheca dakarensis* by its pseudoparenchymatous medulla (Kuang & Xia, 2003) and may, indeed, not even be a member of this species complex.

Comparison between *M. dakarensis* and five other species of *Meristotheca* is shown in Table 2. The thicker blades in our alga may be one of its major diagnostic features, although they do not greatly exceed those recorded for *M. coacta* and *M. papulosa*. Three species, *M. fergussonii*, *M. tobagensis* and *M. papulosa*, have erect rather than decumbent blades, those of *Meristotheca fergussonii* being not only smaller than those of *M. dakarensis*, but also provided with marginal teeth that the Senegalese alga lacks. At any rate, the generic status of *M. fergussonii* is questionable in the continued absence of any reproductive features. *Meristotheca tobagensis* also has dentate margins (Tab. 2), but as no reproductive structures of any sort are known for it its inclusion in *Meristotheca* is highly provisional. *Meristotheca papulosa*, the type species, in addition to being erect produces marginal cystocarps (Gabrielson & Kraft, 1984), the positioning of these structures being different and apparently quite stable in at least some species of the genus (Tab. 2).

Among prostrate to recumbent species, *M. procumbens* comes closest to the habit of *M. dakarensis* but is monoecious rather than dioecious and produces marginal cystocarps. *Meristotheca coacta* has finely divided, imbricating and recumbent thalli that form distinct hummocks (Watt *et al.*, 2003, fig. 3), as well as marginal cystocarps (Tab. 2).

The position of pit-connections between tetrasporangia and their parental cells is a supposedly significant generic feature in the Solieriaceae (Gabrielson & Hommersand, 1982a, b). However, as shown for *Eucheuma serra* (J. Agardh) J. Agardh, tetrasporangial initials are attached by basal pit-connections to their bearing cells and produce a basal protuberance which shifts the initially

Table 2. A comparison of the known species of *Meristotheca*.

	M. coacta	*M.dakarensis*	*M. fergussonii*	*M. papulosa*	*M. procumbens*	*M. tobagensis*
Habit of blades (size)	Prostrate, irregularly dichotomously divided, forming a roundish, depressed knoll-like heap	Decumbent, irregularly dichotomously divided	Erect, flabellate-rounded	Erect (sometimes with prostrate base), irregularly dichotomously divided	Prostrate, irregularly lobed and branched	Erect, marginally irregularly forked
Dimension of blades (length or diameter and thickness)	15-20 cm in diameter and 300-700 µm thick[1]	10-30 cm high and 500-1200 µm thick	Up to 2 cm in diameter[2]	20-30 cm high and 400-900 µm thick[3]	10 cm in diameter and 100-500 µm thick	Up to 6 cm high and 530 µm thick
Widths of major axes or segments	5-10 mm	10-40 mm	Unknown	Up to 50 mm	Unknown	Unknown
Margins of blades	Toothed or fimbriate, with short, blunt, finger-like processes (sometimes entire)	Smooth, or with simple or branched proliferations	Toothed, with laciniate, renulate, or rounded proliferations	Simple to branched, blunt to conical proliferations	Smooth (occasinally toothed -fringed)	Aculeate-dentate above, smooth below
Position of pit-connections between tetrasporangial initials and the parental cells	Unknown	Basal	Unknown	Lateral	Lateral	Unknown
Monoecious or dioecious	Unknown	Dioecious	Unknown	Unknown	Monoecious	Dioecious
Number of cells in carpogonial branches	Unknown	3, occasionally 2 or 4	Unknown	3	3	Unknown
Sterile cells on carpogonial branches	Unknown	Rarely present	Unknown	Occasionally present	Absent	Unknown
Auxiliary cell complex	Unknown	Present	Unknown	Absent	Absent	Unknown
Position of cystocarps	Blade margin[4]	Numerous on dorsal surface (a few on ventral surface and margins)	Unknown	Blade margin	Blade margin	A few on surfaces
Type locality	Yura-jima, Koshiki Islands, Japan	Dakar, Senegal	Sri Lanka	Hodeida, Yemen	Neds Beach, Lord Howe Island, Australia	Man-of-War Bay, Tobago Island
Geographical distribution	NW Pacific	SE Atlantic	Indian Ocean	Red Sea, Indian Ocean, Pacific	SW Pacific	Caribbean Sea
Reference	Okamura, 1930	Present paper	Mazza, 1920	Okamura, 1926 [as *Eucheuma papulosa* (Montagne) Cotton *et* Yendo]; Gabrielson & Kraft, 1984	Gabrielson & Kraft, 1984	Taylor, 1962

1. Thickness taken from lectotype material (Yura-jima, Koshiki Islands, Japan, vii.1919, Herb. K. Okamura in SAP).
2. Thickness is unknown.
3. Thickness taken from our two collections: Ookataura, Hachijo Island, Tokyo, Japan (13.vii.2003, SAP 096627, 096628) and Ishijirogawa, Shikine Island, Tokyo, Japan (26.viii.2003, SAP 09629, 096630)
4. Although Okamura (1930) did not mention cystocarps in the protolog, the lectotype specimen (deposited in SAP) has marginal cystocarps.

basal pit-connection to a lateral position as the sporangium matures (Gabrielson & Kraft, 1984). In another species of *Eucheuma* [*E. isiforme* (C. Agardh) J. Agardh], however, tetrasporangia are laterally cut off from their parental cells at inception (Gabrielson, 1983). Therefore, the position of pit-connections between tetrasporangial initials and the parental cells appears to have more significance at the species rather than the genus level, so that the anomalous basal rather than the usual lateral position (Gabrielson & Kraft, 1984; Gabrielson & Cheney, 1987, as *Meristiella*; Guimarães & Oliveira, 1996, as *Meristiella*) of the pit-connection in *M. dakarensis* is regarded as a species character only.

CONCLUSIONS

We feel that the species referred to as *Meristotheca senegalensis* J. Feldmann by several phycologists (Sourie, 1954; Bodard & Mollion, 1974; Fostier, 1989; Fostier *et al*., 1992; Harper & Garbary, 1997) is likely to be equivalent to our *M. dakarensis* [different from *Sarcodia ceylanica* in the Sarcodiaceae that is procarpic (Liao *et al*., 1993) and no longer even belonging to the same order as *Meristotheca* (Saunders *et al*., 2004)], although absolute certainty is impossible so long as Feldmann's type and/or authentic material remains unlocated. For that reason we propose that the following formal name and description be applied to the recent Senegalese collections analyzed in our study:

Meristotheca dakarensis **Faye *et* Masuda, sp. nov.**

Laminae decumbentes, 10-30 cm altae, 500-1200 µm crassiore in partibus supernis ad infernis, irregulariter dichotome divisae quadriplo ad duodecimplo, segmentis 1-4 cm in latitudine, cum vel sine proliferationibus marginalibus, profunde rubrae rosae vel profunde brunneae rubellae, carnosae, tenices, interne multiaxiales; proliferationes (ubi adsunt), simplices, laciniatae (usque ad 2 mm in longitudine) vel dichotome divisae duplo ad quadriplo, foliaceae (usque ad 7 cm in longitudine). Reproductio more generis. Initium tetrasporangii e cellula parentalia basaliter abscissum; tetrasporangia super partes superas ad medias laminae dispersa, anguste ellipsoidalia, 38-45 µm in longitudine, 15-20 µm diametro. Gametophyta dioecia, structuras reproductivas super partes superas ad medias laminae efferentia; spermatangia ellipsoidalia, 4-5 µm in longitudine, 3-4 µm diametro; ramus carpogonialis plerumque tricellularis interdum bicellularis vel quadricellularis, raro laterale sterili; complexio cellulae auxiliaris adest; protuberationes cystocarpiis numerosae super pagina dorsali (paucae super pagina ventrali et marginibus), 1.5-2.0 mm in altitudine, 1.5-2.8 mm diametro, cystocarpiis uno vel duo 750-850 µm in altitudine, 700-800 µm diametro (cum textura involvente 50-80 µm in crassitudine); catenae carposporangiorum nonramosae vel interdum ramosae semel, ex cellulis usque ad septem constantes.

Blades decumbent, 10-30 cm high, 500-1200 µm thick in the upper to lower portions, irregularly dichotomously divided four to twelve times, with segments 1-4 cm in width, with or without marginal proliferations, deeply rose-red or deeply reddish-brown, fleshy, tough, internally multiaxial; the proliferations (when present), simple, laciniate (up to 2 mm in length) or dichotomously divided two to four times, foliose (up to 7 cm in length). Reproduction as for the genus.

Tetrasporangial initial cut off basally from the parental cell; tetrasporangia scattered over the middle to upper portions of the blade, narrowly ellipsoidal, 38-45 µm in length, 15-20 µm in diameter. Gametophytes dioecious, producing reproductive structures over the middle to upper portions of the blade; spermatangia ellipsoidal, 4-5 µm in length, 3-4 µm in diameter; carpogonial branch usually three-celled occasionally two- or four-celled, rarely with a sterile lateral; auxiliary-cell complex present; cystocarpic protuberances numerous on the dorsal surface (a few on the ventral surface and margins), 1.5-2.0 mm in height, 1.5-2.8 mm in diameter, with one or two cystocarps 750-850 µm in height by 700-800 µm in diameter (including an enveloping tissue 50-80 µm in thickness); carposporangial chains unbranched or occasionally branched once, consisting of up to seven cells.

Holotypus: Cystocarpic specimen deposited in SAP (096664, Fig. 1), Ngor Island, Dakar, Senegal, 10 May 2003, *leg*. G. Dioh Faye.

Etymology: The specific epithet, *dakarenis*, refers to Dakar City in which the algal type locality, Ngor Island, is situated.

Distribution: Endemic to Dakar and its vicinity (Fostier, 1989; Fostier *et al*., 1992, as *Meristotheca senegalensis*).

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