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# A Reassessment and Reclassification of Species in the Genera *Onikusa* Akatsuka and *Suhria* J. Agardh *ex* Endlicher (Gelidiales, Rhodophyta) Based on Molecular and Morphological Data

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The phylogenetic relationships and taxonomic status of the Gelidialean genera *Onikusa* Akatsuka and *Suhria* J. Agardh *ex* Endlicher were examined based on analyses of molecular and morphological characters. The DNA sequences of *rbcL* from multiple specimens of all *Onikusa* and *Suhria* species were included in analyses of 38 Gelidialean taxa having the *Gelidium*-type female reproductive and cystocarp systems. Six well-supported species clades were resolved in these analyses. Two species of *Onikusa* and *Suhria vittata* (Linnaeus) Endlicher were resolved within the '*Suhria* clade', and *Onikusa japonica* (Harvey) Akatsuka was resolved within the '*Gelidium chilense* clade.' *Gelidium* Lamouroux was paraphyletic with respect to *Onikusa* and *Suhria*. Evidence for a turf-form ecotype of *Onikusa pristoides* was found. Examination of species resolved within the '*Suhria*,' '*Gelidium chilense*' and '*G. coulteri* clades' revealed that the medullary structure type was not a synapomorphy for these clades. Based on these new and previous findings, the reversion of *Suhria vittata*, *Onikusa pristoides* (Turner) Akatsuka, and *Onikusa japonica* to *Gelidium*, and the new combinations *Gelidium vittatum* (Linnaeus) Kützing forma *laceratum* (Grunow) D.W. Freshwater and *Gelidium foliaceum* (Okamura) E.M. Tronchin are proposed.

## Introduction

Phylogenetic reconstructions based on a variety of DNA sequence data have shown that the red algal order Gelidiales is taxonomically problematic (Freshwater *et al.* 1995, Bailey and Freshwater 1997, Freshwater and Bailey 1998, Shimada *et al.* 1999). Separate and combined analyses of chloroplast encoded *rbcL*, nuclear encoded SSU and nuclear encoded LSU sequences have resolved four major clades within the Gelidiales that correspond to specific ontogenetic patterns of the female reproductive and cystocarp systems (Bailey and Freshwater 1997, Freshwater and Bailey 1998, Shimada *et al.* 1999). Three of these major clades correspond to the genera *Gelidiella* Feldmann *et* Hamel, *Pterocladia* J. Agardh and *Pterocladiella* Santelices *et* Hommersand. Species in the fourth major clade have a '*Gelidium*-type' female reproductive and cystocarp system. This major clade contains the majority of Gelidialean taxa and includes species in the genera *Acanthopeltis* Okamura *in* Yatabe (including *Yatabella* Okamura), *Capreolia* Guiry *et* Womersley, *Gelidium* Lamouroux, *Onikusa* Akatsuka, *Ptilophora* Kützing (including *Beckerella* Kylin) and *Suhria* J. Agardh *ex* Endlicher. Eight subclades (the species complexes of Freshwater *et al.* 1995) have been resolved within this major clade but they do not correspond to the currently accepted generic designations (Freshwater *et al.* 1995, Shimada *et al.* 1999); most notably the ordinal type, *Gelidium*, is paraphyletic. Revision of the generic assignment of some species is necessary to attain a phylogeny-based natural classification of the Gelidiales.

It may be argued that the strong molecular support for the subclades within the 'Gelidium-type' female reproductive and cystocarp system clade should lead to the recognition of these subclades at the genus level. We believe that morphological/ontogenetic synapomorphies correlating with the molecular clades are required before recognition at the genus level is warranted. Numerous morphological criteria for distinguishing genera and species have been proposed for the Gelidiales including: 1) rhizine distribution (Feldmann and Hamel 1934, Okamura 1934), 2) basal bending of indeterminate branches (Stewart 1976), 3) arrangement of surface cortical cells (Akatsuka 1981, 1986a, 1986b), 4) apical architecture (Rodríguez and Santelices 1987), 5) medullary structure (Rodríguez and Santelices 1996), and 6) ontogenetic patterns of the female reproductive and cystocarp systems (Hommersand and Fredericq 1996, Santelices and Hommersand 1997). Only the ontogenetic patterns of female reproductive and cystocarp systems have proven reliable when critically examined, but the medullary structure has not been thoroughly tested.

For example, detailed studies of the female reproductive system and cystocarp development by Santelices (1991a, 1991b) and Hommersand and Fredericq (1996) led to the proposal of the genus *Pterocladiella* for four species formerly placed in *Pterocladia* and *Gelidiella* (Santelices and Hommersand 1997). Subsequent examination of additional species has supported the establishment of *Pterocladiella* and resulted in the transfer and description of additional species in this genus (Santelices 1997, 1998, Thomas and Freshwater 2001). Likewise, molecular analyses of species transferred to *Pterocladiella* strongly resolve them as a monophyletic clade supporting the distinction of this genus as a natural taxonomic group (Freshwater and Bailey 1998, Shimada *et al.* 1999, Thomas and Freshwater 2001, Freshwater *et al.* unpublished).

In this paper we continue the development of a natural classification system for the Gelidiales by revising the status of *Onikusa* and *Suhria*. This revision is based on past and new molecular and morphological analyses that include 8 previously unpublished *rbcL* sequences and a critical examination of medullary structure characteristics in a limited number of species. The relationship of *Onikusa foliacea* (Okamura) R. E. Norris and a reported turf form of *O. pristoides* (Turner) Akatsuka is also examined.

## Material and Methods

### Molecular analyses

Eight new and 30 previously published *rbcL* sequences were analyzed in this study. GenBank accession numbers for all analyzed sequences and the collection locations for the taxa from which they were generated are listed in Table I. The new *rbcL* sequences came from specimens collected at field sites and dried using silica gel desiccant (Chase and Hills 1991). Total genomic DNA was extracted from 10–30 mg of tissue following the protocol of Hughey *et al.* (2001). Amplification and sequencing of *rbcL* was as described in Thomas and Freshwater (2001). The sequences of primers used in this study are presented in Freshwater and Rueness (1994). Sequence data were compiled and edited using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA). Characteristics of the aligned sequence data were determined using PAUP (v. 4.0b8, Swofford 2001) and MacClade (v. 4.0, Maddison and Maddison 2000).

Phylogenetic trees were generated using maximum parsimony, distance, and maximum likelihood methods. Parsimony trees were generated with a heuristic search scheme of 1000 random sequence additions, MULPARS, STEEPEST DESCENT, and the tree bisection reconnection (TBR) branch-swapping algorithm. A second search that allowed branch swapping on all trees up to five steps less parsimonious than trees found in the initial search was done as a further check for islands of minimal trees (Maddison 1991). Bootstrap analyses (Felsenstein 1985) consisted of 1000 replications of heuristic searches using simple sequence addition, MulTrees, STEEPEST DESCENT, and TBR branch swapping. For the distance analysis a neighbor joining tree (Saitou and Nei 1987) was constructed from Tamura-Nei distances (Tamura and Nei 1993). Distance bootstrap analyses consisted of 1000 replications of neighbor joining tree construction with Tamura-Nei distances. Before performing maximum likelihood analyses the transition:transversion ratio (tn:tv) that maximized the log-likelihood value was calculated by plotting a range of tn:tv against the corresponding log-likelihoods for the distance tree. The resulting value (tn:tv = 3.2) was subsequently used with empirical base frequencies in 10 separate likelihood analyses of random sequence addition, MulTrees, and the TBR branch swapping algorithm. Maximum likelihood bootstrap values were calculated from 100 replications of random addition of sequences, MulTrees, and TBR branch swapping.

### Morphological observations

Medullary structure characters defined in Rodríguez and Santelices (1996) were revisited using nine species in the Gelidiaceae. Observations were made of the medullary structure of *Suhria vittata* (Linnaeus) Endlicher (Oudekraal, RSA), *Onikusa foliacea* (East London, RSA), *O. pristoides* (Glencairn, RSA), *O. japonica* (Harvey) Akatsuka (Keelung, North Taiwan), *Gelidium micropterum* Kützing (Glencairn, RSA), *G. microdonticum* Taylor (Cahuita, Costa Rica), *G. capense* (Gmelin) Silva (Glencairn, RSA), *G. coulteri* Harvey (Stillwater Cove, Pacific Grove, California, USA) and *G. chilense* (Montagne) Santelices *et* Montalva (Tongoy Bay, Coquimbo, Chile). Silica gel dried and preserved specimens (fixed in 5–10 % Formalin-seawater at the time of collection) where used. Dried specimens were hydrated in seawater, or 5 % Formalin-seawater. Following the method described by Rodríguez and Santelices (1996), paradermal, longitudinal and cross sections were cut by hand or with a freezing microtome from apical tips 0.5 cm in length. All specimens were stained with 1 % aniline blue stain and fixed in 50 % Karo™ solution. Specimens were observed and photographed using a Nikon Coolpix 990 digital camera (Nikon Corp., Tokyo, Japan) mounted on a Leitz Dialux 20 EB compound microscope (E. Leitz, Inc., Wetzlar, Germany).

## Results

### Molecular analyses

A *rbcL* data set of 38 taxa was analyzed in this study. The first 67 base pairs (bp) of the 1467 bp gene were

Table I. Collection location and GenBank accession numbers for taxa included in *rbcL* sequence analyses. Newly published sequences are indicated by an *.

| Taxa | Collection location | GenBank accession |
|---|---|---|
| *Gelidium allanii* Chapman | Doubtless Bay, North Island, New Zealand[1] | L22458 |
| *G. americanum* (Taylor) Santelices | Radio Island, Carteret Co. NC, USA[2] | L22459 |
| *G. canariensis* (Grunow) Seoane-Camba | Puerto de la Cruz, Tenerife, Canary Islands[3] | L22460 |
| *G. capense* (Gmelin) Silva | False Bay, Cape Peninsula, South Africa[4] | L22461 |
| *G. chilense* (Montagne) Santelices *et* Montalva | Tongoy Bay, Coquimbo, Chile[5] | AF305800 |
| *G. coulteri* Harvey | Balboa Peninsula, Orange Co., CA, USA[2] | U00105 |
| *G. crinale* (Turner) Gaillon | Masonboro Inlet, New Hanover Co., NC, USA[2] | U00981 |
| *G. crinale* | Awhai Is., Hyogo Pref., Japan (Shimada *et al.* 1999) | AB017679 |
| *G. floridanum* Taylor | Sebastian Inlet, Indian River Co., FL, USA[2] | U00107 |
| *G. latifolium* (Greville) Bornet *et* Thuret | Plouguerneau, Brittany, France[6] | U00112 |
| *G. microdonticum* W.R. Taylor | Cahuita, Limón, Costa Rica[2] | AF305799 |
| *G. micropterum* Kützing | Kommetjie, Cape Peninsula, South Africa[4] | U00446 |
| *G. pacificum* Okamura | Amatsukominato, Chiba Pref., Japan[7] | U16832 |
| *G. pluma* Loomis* | Hawai'i, Hawaiian Islands[8] | AF501288 |
| *G. pulchellum* (Turner) Kützing | Aramar, Asturias, Spain[9] | U01822 |
| *G. pusillum* (Stackhouse) Le Jolis | Cancale, Brittany, France[9] | U01000 |
| *G. pusillum* | Fedje, Hordaland, Norway[9] | U00999 |
| *G. 'pusillum'* | Puerto de la Cruz, Tenerife, Canary Islands[9] | U01003 |
| *G. 'pusillum'* | Praia de Peruibe, Estado de Sao Paulo, Brazil[10] | U01004 |
| *G. 'pusillum'* | Solano Beach, San Diego Co., CA, USA[11] | U00984 |
| *G. 'reptans'* (Suhr) Kylin | Natal, South Africa (culture #0962)[12] | AF305798 |
| *G. rex* Santelices *et* Abbott | Tongoy Bay, Coquimbo, Chile[5] | AF305801 |
| *G. robustum* (Gardner) Hollenberg *et* Abbott | Dana Point, Orange Co., CA, USA[2] | U01041 |
| *G. serrulatum* J. Agardh | Mochimo, Sucre, Venezuela[2] | U01042 |
| *G. sesquipedale* (Clemente) Thuret *in* Bornet *et* Thuret | Aramar, Asturias, Spain[3] | L22071 |
| *G. vagum* Okamura | Jodogahama, Iwate Pref., Japan (Shimada *et al.* 1999) | AB017680 |
| *'Onikusa' foliacea* (Okamura) R.E. Norris* | Port Edward, KwaZulu-Natal Prov., South Africa[13] | AF501284 |
| *'O.' foliacea** | East London, Eastern Cape Prov., South Africa[13] | AF501286 |
| *'O.' foliacea** | Breezy Point, Eastern Cape Prov., South Africa[13] | AF501285 |
| *'O.' japonica* (Harvey) Akatsuka* | Keelung, Taiwan[14] | AF501287 |
| *'O.' japonica* | Shimoda, Shizuoka Pref., Japan (Shimada *et al.* 1999) | AB017676 |
| *'O.' sp.* | GenBank submission by Shimada *et al.* | AB017677 |
| *'O.' pristoides* (Turner) Akatsuka | False Bay, Western Cape Prov., South Africa[11] | U01044 |
| *'O.' pristoides** | Port Edward, KwaZulu-Natal Prov., South Africa[13] | AF501282 |
| *'O.' pristoides* 'turf form'* | Kidds Beach, Eastern Cape Prov., South Africa[13] | AF501283 |
| *Ptilophora scalarimosa* (Kraft) Norris | Bulusan, Sorsogon Province, Luzon, Philippines[15] | AF305804 |
| *Suhria vittata* (Linnaeus) J. Agardh | Kommetjie, Western Cape Prov., South Africa[11] | U00112 |
| *S. vittata** | Lüderitz, Namibia[16] | AF501289 |

Samples for DNA extraction provided by: [1]W. Nelson, [2]D.W. Freshwater, [3]J. Rico, [4]J. Bolton, [5]M.E. Edding, [6]J. Cabioc'h & M.H. Hommersand, [7]M. Yoshizaki, [8]K.J. McDermid, [9]culture of J. Rueness & S. Fredriksen, [10]M. Cordeiro-Marino, [11]M.H. Hommersand, [12]culture of Rico & Guiry, [13]E.M. Tronchin, [14]S.M. Lin, [15]L. Liao, [16]A.T. Critchley.

excluded from all analyses because a majority of the analyzed taxa were missing data for these sites. Of the remaining 1400 sites, 433 were variable and 312 were parsimony informative. The data set had an overall AT base bias although base usage varied considerably with codon position (Table II). The tn:tv for the data set was 3.4 and there was a bias of 324 pyrimidine transition substitutions to 207 purine transition substitutions. Based on these findings, the Tamura-Nei correction was used when calculating all distances.

Table II. Nucleotide base usage (%) at different codon positions in the *rbcL* data set for 38 Gelidiales taxa.

| Codon position | A | C | G | T |
|---|---|---|---|---|
| First | 26.3 | 16.3 | 38.0 | 19.4 |
| Second | 30.2 | 23.4 | 16.7 | 29.7 |
| Third | 36.0 | 10.7 | 8.9 | 44.4 |
| All sites | 30.8 | 16.8 | 21.2 | 31.2 |

The topologies derived from all three tree-building methods were nearly identical. Parsimony analyses resulted in three minimal trees of 991 steps (all sites) and consistency indices (CI) of 0.45 (informative sites only) (Fig. 1). The distance and maximum likeli-

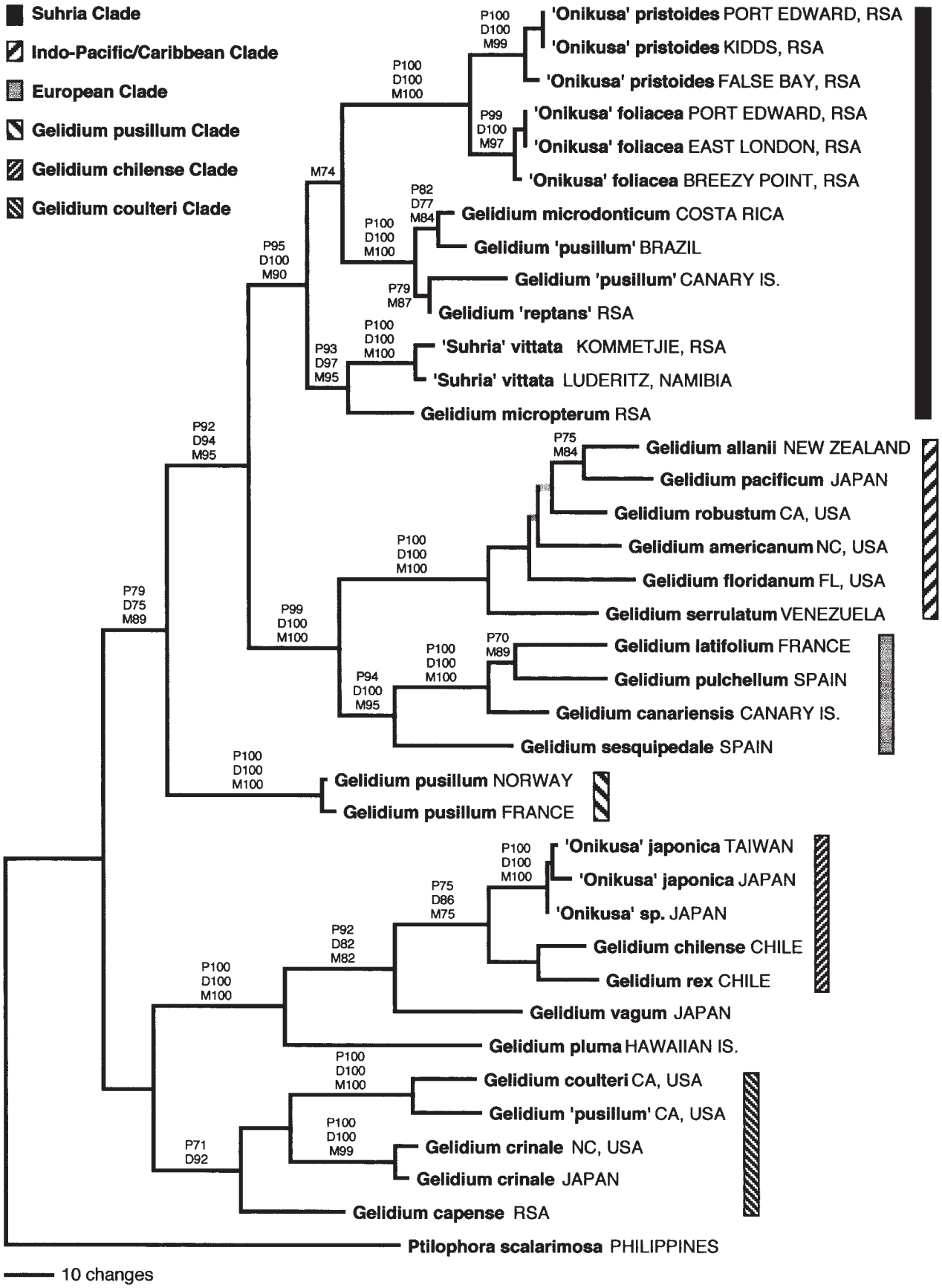


Fig. 1. One of three minimal parsimony trees (L = 991, CI = 0.45) resulting from analyses of *rbcL* sequence data from 38 Gelidiales taxa. Branches not present in all minimal trees are stippled. Bootstrap proportion values for parsimony (P), distance (D) and maximum likelihood (M) analyses are presented on branches when greater than 70.

hood topologies differed only in the relationship of *Gelidium chilense* to *G. rex* Santelices *et* Abbott.

Samples of *Onikusa pristoides*, *O. foliacea* and *Suhria vittata* were resolved within a strongly supported clade (bootstrap proportion [BP] = parsimony [P] 95, distance [D] 100, maximum likelihood [M] 90) referred to as the '*Suhria* species complex' by Freshwater *et al.* (1995). Sequences of *S. vittata* from Kommetjie, South Africa and Lüderitz, Namibia varied by 0.48 %. *Gelidium micropterum* had a strongly supported sister relationship to *Suhria vittata* (BP = P93, D97, M95). Distances between sequences of *Onikusa pristoides* from Port Edward, Kidds Beach and False Bay, South Africa ranged from 0.0 to 0.50 %. The turf form sample from Kidds Beach had an identical sequence to the sample from Port Edward. Similarly, distances between sequences of *O. foliacea* from Port Edward, East London and Breezy Point, South Africa ranged from 0.0 to 0.22 %. Samples of *O. pristoides* (BP = P100, D100, M99) and *O. foliacea* (BP = P99, D100, M97) were strongly supported as distinct monophyletic clades that are sister in the *rbcL* tree (BP = P100, D100, M100). Sequences for *O. japonica* from Japan and Taiwan were strongly resolved as a monophyletic clade (BP = P100, D100, M100) within a clade separate from that which includes *O. pristoides* and *O. foliacea*. The strongly supported clades containing the *Onikusa* and *Suhria* species also include species of *Gelidium* (Fig. 1).

## Morphological analyses

No differences in medullary cell dimensions or organization were found between cells hydrated with seawater or a 5 % Formalin-seawater solution. Similarly, no differences were found when comparing sections mounted in seawater to sections mounted in a 50 % Karo™ solution.

Paradermal sections of *Suhria vittata* revealed a loose mesh of narrow, elongated medullary cells with

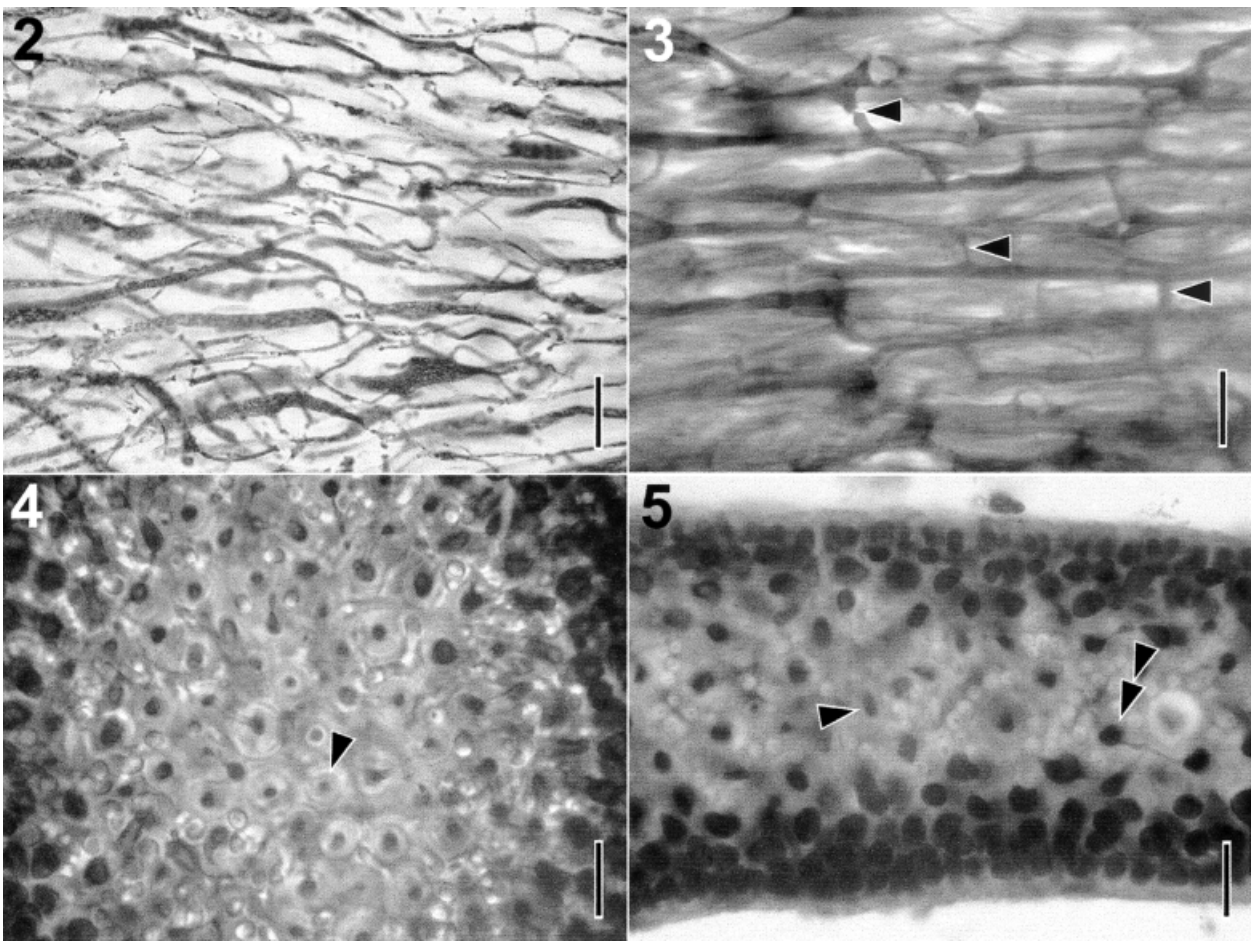


Fig. 2. Subapical paradermal section of *Suhria vittata* showing a loose mesh of medullary cells. Scale = 50 µm. Fig. 3. Subapical paradermal section of *Onikusa pristoides* showing a regular network of medullary cells and H-shaped intercellular connections (arrowheads). Scale = 20 µm. Fig. 4. Subapical cross section of *Gelidium coulteri* showing cylindrical medullary cells (arrowhead) and rhizines concentrated in the inner cortex. Scale = 20 µm. Fig. 5. Subapical cross section of *Gelidium chilense* showing cylindrical cells (double arrowhead) and few scattered subcylindrical medullary cells (single arrowhead). Scale = 20 µm.

lateral and terminal connections that ran in various directions (Fig. 2). Medullary cells were 2–9 μm in diameter (with within-cell variation of ± 1.5 μm) and 35–185 μm in length.

Paradermal sections of *Onikusa pristoides* revealed a very regular medullary structure composed of closely associated cells oriented parallel to one another. The regular structure was enhanced by the presence of numerous lateral H-shaped intercellular connections between adjacent cells (Fig. 3). Medullary cells were 2–7 μm in diameter (with within-cell variation of 1–2.5 μm) and 24–109 μm in length. Rhizines were abundant in the inner cortex, especially in the alae, but absent in the medulla of the midrib.

*Gelidium coulteri* had closely apressed cylindrical cells with rhizines concentrated in the inner cortex (Fig. 4). *Gelidium capense* had predominantly cylindrical cells, but flattened cells were also encountered. *Onikusa japonica* had mostly cylindrical cells in subapical sections with flattened ribbon-like cells occurring subcortically and scattered throughout the medulla. *Gelidium chilense* had mostly cylindrical cells subapically with relatively few scattered subcylindrical cells (Fig. 5). *Gelidium capense* and *Onikusa japonica* had rhizines concentrated in the inner cortex whereas rhizines occurred throughout the medulla of *Gelidium chilense*.

All nine species had a medullary structure that could be described as a mesh, differing in various degrees of looseness and regularity. *Onikusa pristoides*, *Gelidium coulteri* and *G. chilense* had the tightest and most regular medullary structure. Evidence of varying degrees of twisting in medullary cells was found in all the species. The presence of H-shaped intercellular connections (Fig. 2) was noted in *Onikusa pristoides*, *O. japonica*, *Gelidium micropterum*, *G. capense*, *G. coulteri*, *G. chilense* and *Suhria vittata* (though not common in the latter).

## Discussion

### Molecular analyses

Analyses of *rbcL* sequence data support the recognition of *Onikusa foliacea*, *O. japonica*, *O. pristoides* and *Suhria vittata* as discrete species. Percent sequence divergence between specimens of these species were low (≤ 0.5 %) despite the considerable geographical distance between sample locations. The *rbcL* phylogeny presented here (Fig. 1) is congruent with the results of previous *rbcL* analyses (Freshwater *et al.* 1995, Shimada *et al.* 1999) in resolving a number of well-supported subclades within the major clade of species with the 'Gelidium-type' female reproductive and cystocarp system. Two of the three species currently placed in *Onikusa* are resolved along with *Suhria vittata* and five species of *Gelidium* within what has been referred to as the '*Suhria* clade' or '*Suhria* species complex.' The remaining species of *Onikusa*, *O. japonica*, is resolved within a separate group of species that is referred to as the '*Gelidium chilense* clade' (Fig. 1). As in all previous analyses with *rbcL* and other DNA sequences (Freshwater *et al.* 1995, Bailey and Freshwater 1997, Freshwater and Bailey 1998, Shimada *et al.* 1999, Thomas and Freshwater 2001) *Gelidium* is paraphyletic with respect to *Onikusa* and *Suhria*.

### Medullary cell structure

Rodríguez and Santelices (1996) described six types of medullary structure in a study of 21 Gelidiales species. Medullary cell organization, morphology and dimension, as well as the distribution of rhizines defined these types. The characterization of medullary types was restricted to apical and subapical portions of erect axes, with the rationale that in this region cells are becoming a distinctive tissue still unmodified by age and environment and should not be variable in form and shape. Some of the defined medullary structure types were observed in taxa that had previously been resolved in specific species clades by *rbcL* analyses (Rodríguez and Santelices 1996: table 3). In the present study medullary structure type was examined in multiple species from the *rbcL*-defined *Suhria*, *Gelidium coulteri* and *G. chilense* species clades to determine if the *Onikusa pristoides*, *Gelidium coulteri* and *G. chilense* medullary types are synapomorphic for these species clades respectively.

The '*Onikusa pristoides* type' was described as a loose mesh of narrow, elongated medullary cells with lateral and terminal connections that run in various directions. Subapical cells were 50–90 μm long and 2–4 μm in diameter. The '*Gelidium coulteri* type' was described as a loose composition of flattened (ribbon-like) cells running parallel to the axis, with various degrees of undulation and twisting. Rhizines occurred throughout the medulla of both types. Medullary cells of the '*Gelidium chilense* type' were described to be cylindrical or subcylindrical apically and elliptical or compressed subapically, exhibiting varying degrees of twisting, with H-shaped intercellular connections in paradermal view.

The medullary structure of *Suhria vittata* was found to fit the general description of the '*Onikusa pristoides* type', but it has much larger cell dimensions than the dimensions defining this type. The medullary structure of the *O. pristoides* specimen observed in this study was very regular and did not fit the description of the '*Onikusa pristoides* type'. Neither did the cell dimensions and the distribution of rhizines agree with the description of this type. Figure 3.5c, p. 25 in Carter (1986) also shows a very regular medullary structure with wide intercellular spaces, parallel rows of cells and H-shaped intercellular connections. Interspecific variation in the medullary structure was also found among *O. foliacea*, *Gelidium micropterum* and *G. microdonticum*,

three other species resolved in the '*Suhria* clade'. The lack of a consistent medullary structure type in the species of the *Suhria* clade demonstrates that these characters can not be used as a morphological synapomorphy for the clade.

Given that *Onikusa japonica* is resolved in an *rbcL* clade with *Gelidium chilense*, the medullary structures of *Onikusa japonica*, *Gelidium chilense* and two species of the related '*Gelidium coulteri* clade', *G. coulteri* and *G. capense*, were investigated. The medullary structures of all four species disagreed with the description of the '*Gelidium coulteri* type', given the presence of both cylindrical and flattened (or subcylindrical) cells and an even distribution of rhizines throughout the medulla. The medullary structure of *Onikusa japonica* did not fit the '*Gelidium chilense* type' due to the occurrence of both cylindrical and flattened ribbon-like cells subapically. The *G. chilense* examined here did not agree entirely with the '*Gelidium chilense* type' as cylindrical cells were found throughout the length of the apical tip, even up to 1 cm below the apex. Neither the *G. coulteri* nor *G. chilense* type medullary structures defined by Rodríguez and Santelices (1996) adequately define the taxa studied here.

Medullary structure type, rhizine distribution and cell dimension were found to be inconsistent between species in the 3 examined *rbcL*-defined clades. Therefore these characters can not be used as synapomorphies for these species clades. Additional study of these characters is needed to determine their potential utility as synapomorphies for other species and species clades.

### *Onikusa*

Akatsuka (1986b) erected the genus *Onikusa* for two species formerly included in *Gelidium*, *Onikusa pristoides* from South Africa (type species) and *O. japonica* from East Asia. Norris (1992a) later described a third species, *O. foliacea*, for a taxon from Japan and South Africa.

Akatsuka (1986b) assigned members of the Gelidiales to groups based on various combinations of morphological characters. *Onikusa pristoides* and *O. japonica* were removed from *Gelidium* and placed in the '*Suhria*' group (including *Suhria*, and *Porphyroglossum* Kützing) based on the shared occurrence of surface cortical cells in tetrads in the middle of the main thallus axis (Akatsuka 1986b). *Onikusa*, *Porphyroglossum* and *Suhria* were treated as separate genera due to other macro- and micro-morphological differences. Algal taxonomists however, have not uniformly accepted *Onikusa* (Stegenga *et al.* 1997, Yoshida 1998).

Rodríguez and Santelices (1988) found tetrads of cortical cells to be common in species of *Gelidium* and *Pterocladia* (including *Pterocladiella*) in apical portions of the thallus and that they can occur elsewhere along the axis as well. The occurrence of cortical cells in tetrads is indicative of areas where cell division is still common, and the distance between cells and cell shape have not been modified by growth (Santelices 1988, Norris 1992a). Akatsuka (1986b) supported the use of surface cell morphology to delimit this genus with various vegetative diagnostic characters of the middle region of the main axis. These characters however, show much overlap with other genera such as *Ptilophora*. One of the characters used, the distribution of proliferations or serrulae, is environmentally variable and the presence of surface proliferations can be modified by injury or epiphytic load (Santelices 1990).

Norris (1992a) emended the description of *Onikusa* to highlight a combination of characters in *O. pristoides* and *O. foliacea* he believed were important in delimiting this genus, i.e. the tendency to branch ramisympodially (thought to be a primitive form of ramisympodial branching with the major branches usually being produced adventitiously and emerging from the midrib) and the extensive indeterminate system of prostrate branches. Norris was unsure of the presence of this combination of characters in *O. japonica*, but the examination of specimens shows that it has the same tendency to produce major branches adventitiously from the midrib and also possesses an extensive system of prostrate branches (Tronchin pers. comm.). However, *O. japonica* normally exhibits irregularly to alternately bipinnate branching and there is no mention of ramisympodial branching in the descriptions of this species (Harvey 1859, Akatsuka 1986b). Likewise, the ramisympodial tendency of branching in *O. pristoides* appears to be the exception rather than the rule and is most often an artifact caused by injury to the dominant axis stunting its growth and allowing a lateral to overtop it and assume secondary dominance. Overtopping due to injury of dominant axes is not uncommon and is seen throughout *Gelidium*. The system of indeterminate prostrate branches is also present in most *Gelidium* species though not always as extensive as in *Onikusa pristoides*. Consequently, this combination of characters is not unique to *Onikusa*.

Morphological characters as well as *rbcL* sequence data indicate that *Onikusa japonica* is not closely related to the other *Onikusa* species. The tetrasporophyte generation of *O. japonica* always produces tetraspores (Akatsuka 1983) whereas that in *O. pristoides* produces bispores (Carter 1985), and the characteristic serrulae of *O. pristoides* do not occur in *O. japonica* (Akatsuka 1983). Analyses of the *rbcL* sequence data resolve *Onikusa* species in two different strongly supported clades that are well separated in the *rbcL* phylogeny (Fig. 1). The polyphyly of species currently included in *Onikusa* and the lack of morphological synapomorphies for any combination of *Onikusa* species indicates that it is an artificial assemblage and that it should be reincorporated into *Gelidium*.

Norris (1992a) proposed the name *Onikusa foliacea* to describe an alga forming a mostly monospecific turf, documented to occur along the central and southern KwaZulu-Natal coastline in South Africa. Carter (1986) mentioned a similar turf alga that occurred in his study sites along the Eastern Cape Province coastline of South Africa. Carter suggested that it was an ecotype of *O. pristoides* or at most a variety. It has since been unclear whether the turf investigated by Carter was *O. foliacea* or whether there is indeed a turf form of *O. pristoides*. Carter mentioned that both the large and turf thalli had tooth-like serrulae along the margins of their flattened fronds, a character particular to *O. pristoides* and not *O. foliacea* (Norris 1992b).

The turf form specimen of *O. pristoides* from Kidds Beach in Eastern Cape Province was found to have an identical *rbcL* sequence to one of the large form *O. pristoides* specimens from Port Edward in the KwaZulu-Natal Province. In contrast, the *O. pristoides* and *O. foliacea* specimens from Port Edward varied by > 2.0 %. These data as well as the resolution of separate monophyletic clades corresponding to *O. pristoides* and *O. foliacea* in phylogenetic analyses clearly show that a turf form (ecotype) of *O. pristoides* exists. This taxon is distinguishable by its midrib in well-developed blades and tooth-like marginal serrulae in contrast to *O. foliacea*, which has entire to erose to crispate margins.

### *Suhria*

*Suhria* is currently a monotypic genus. *Suhria vittata* has had a long history of nomenclatural changes, having been placed in *Sphaerococcus* (C.A. Agardh 1822: 233), *Phyllophora* (Greville 1830: 56), *Gelidium* (Kützing 1843: 407), *Dawsonia* (Bory *in* Bélanger 1834:171) and even referred to as a different genus and species on occasion, i.e. *Fucus ornatus* Thunberg (Thunberg 1794: 181 – in part; 1823: 753 – in part), *Fucus ciliatus* Thunberg (Thunberg 1794: 181 – in part; 1823: 753 – in part; Esper 1797–1799), *Fucus caulescens* Gmelin (Gmelin 1768: 173) and *Delesseria caulescens* Lamouroux (Lamouroux 1813: 38) (Anderson 1994). J. Agardh (1842) first proposed *Suhria* as a nomen nudum based on *Phyllophora vittata* Greville 1830, after which Endlicher (1843) provided the first diagnosis of the genus. During the eighteenth century seven other species were included in *Suhria*, but all were later removed. The diagnosis of *Suhria* in Endlicher (1843) was heavily weighted on the reproductive proliferations issuing from the blade and was very general in its description. The structures described occur throughout the Gelidiaceae. Agardh (1823) described the holdfast of *S. vittata* (as *Sphaerococcus vittata* C. Agardh) as a 'radix callosa', or callose root, hinting at its discoid nature. Fan (1961) maintained the generic status of *Suhria* based on the presence of the discoid holdfast being unique within the Gelidiales, but this may alternatively be considered simply a species specific character state.

*Suhria vittata* is resolved within a strongly supported clade of *Gelidium* species in *rbcL* analyses (Fig. 1), and the presence of a discoid holdfast is the only character state unique to this taxon. Given this information, the maintenance of *Suhria* as a monotypic genus is not warranted and it should be reincorporated into *Gelidium.*

### Synapomorphies for a '*Suhria* clade'?

If a morphological synapomorphy for the '*Suhria* clade' were found the clade could be recognized at the genus level, in which case the name *Suhria* would have priority and be applied to all of the species within the clade. Of the characters that have been proposed in the past, only bispore production and the nature of gonimoblast/nutritive filament cell fusions remain as possible morphological synapomorphies for the '*Suhria* clade.'

Bispores have been reported to occur in four of the eight species that are resolved in this clade, i.e. *Onikusa foliacea*, *O. pristoides*, *Suhra vittata* and *Gelidium micropterum*. The species for which the presence of bispores has not been reported may not have been studied with the specific objective of looking for bispores.

Freshwater *et al.* (1995) suggested that the presence of a large post-fertilization fusion cell could be another possible synapomorphy. It was stated that Fan (1961) and Hommersand and Fredericq (1990) demonstrated that the fusion of the carpogonium with adjoining cortical cells results in the formation of a large fusion cell, a feature that could be a synapomorphy for the *Suhria* complex. However, Fan (1961) reported the occurrence of fusion cells in all members of the Gelidiales that were investigated: in addition to *S. vittata*, he found them to occur in *Gelidium robustum* (Gardner) Hollenberg *et* Abbott (as *G. cartilagineum* var. *robustum* Gardner), *Onikusa japonica* [as *G. japonicum* (Harvey) Okamura], *Ptilophora subcostata* (Okamura) Norris [as *Beckerella subcostata* (Okamura) Kylin] and *P. pinnatifida* (J. Agardh) Norris [as *Beckerella pinnatifida* (J. Agardh) Kylin]. Although Fan (1961) reported that the cystocarp development was very similar in the species he studied, there may be slight differences in the development of the fusion cell and gonimoblast that he did not document. Hommersand and Fredericq (1990) reported that a possible difference between *Gelidium* and *Suhria* cystocarp development is that terminal gonimoblast cells either fuse specifically with the terminal cells of the nutritive filaments, as in *Gelidium pteridifolium* Norris, Hommersand *et* Fredericq, or randomly with terminal and intercalary cells, as in *S. vittata*. It is most probable that this difference is only species specific and it must be studied in additional species before being considered as a synapomorphy for the '*Suhria* clade'.

Santelices (1999) has recently reported 4 different patterns of carposporangia production in a study of 12 Gelidiales species. These patterns varied in the size of the fusion network, shape of the carposporangia-initiating gonimoblast cell, and the arrangement and way carposporangia are produced. One of the examined species was *Onikusa pristoides* but the carposporangia-production pattern ascribed to this species was also found in *Gelidium chilense*, *G. rex*, and *G. pluma* Loomis. These four species are resolved in three separate species clades in molecular analyses (Fig. 1, and Freshwater unpublished analyses). Although the *Onikusa pristoides* pattern of carposporangia production is not a synapomorphy for the *Suhria* clade, further investigation of the characteristics described by Santelices (1999) may reveal synapomorphies for other *Gelidium* species clades.

## Conclusion

Maintaining *Suhria* and *Onikusa* as genera has been shown to be untenable. There are two possible synapomorphies for the '*Suhria* clade' that could lead to the continued use of *Suhria*, however, the presence of either synapomorphy in the *Suhria* clade and absence in the other *rbcL* clades has yet to be confirmed. Before the *Suhria* clade could be recognized at the generic level, however, the clades occurring basal to the *Suhria*-clade in the *rbcL* phylogeny would have to be recognized as genera in order to maintain monophyly in the tree. There are taxonomic problems in these basal clades that have yet to be resolved, therefore, it would seem reasonable to work with the information at hand to continue the process of revising the classification of the Gelidiales so that it is practicable. Since *Gelidium* is paraphyletic with respect to *Suhria* and *Onikusa*, these two genera should be reincorporated into *Gelidium*. The following taxonomic changes are thus proposed.

*Suhria vittata* will revert to *Gelidium vittatum* (Linnaeus) Kützing (1843: 407). A new combination is proposed for *Suhria vittata* forma *lacerata*.

*Gelidium vittatum* (Linnaeus) Kützing forma *laceratum* (Grunow) D. W. Freshwater comb. nov. Basionym: *Suhria vittata* forma *lacerata* Grunow (1867: 81). Type locality: St. Paul Island, sin typo.

*Onikusa pristoides* will revert to *Gelidium pristoides* (Turner) Kützing (1843: 407). A new combination is proposed for *Onikusa foliacea*.

*Gelidium foliaceum* (Okamura) E.M. Tronchin comb. *et* stat. nov. Basionym: *Gelidium pusillum* forma *foliaceum* Okamura (1934: 51, pl. 17, figs 3–4, pl. 31). Type locality: Japan, on barnacles at Shisô-dima, Seto, Prov. Kii, Okamura s.n. (iso., LD) Synonym: *Onikusa foliacea* (Okamura) R.E. Norris (1992a: 169, figs 7–10 [including Indian Ocean record]); R. Norris (1992b: 21, figs 10, 11).

*Onikusa japonica* will revert to *Gelidium japonicum* (Harvey) Okamura (1901: 57–60).

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