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Research Article

New insights into the phylogeny of *Spyridia* (Ceramiales, Rhodophyta) species with uncinate spines, focusing on the structure of determinate branches

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ABSTRACT

Twenty-one samples belonging to species of *Spyridia* Harvey with uncinate (= hook-shaped) spines on determinate lateral branches were investigated for their morphology with a focus on the structure of determinate branches, and for comparative chloroplast-encoded *rbc*L and nuclear LSU rDNA sequence analysis to elucidate their taxonomy and phylogeny. Currently, four *Spyridia* species with uncinate spines are recognized worldwide: *S. alternans*, *S. cupressina*, *S. horridula* and *S. hypnoides*. Of them, *S. hypnoides* has been recognized as the most common species with uncinate spines. In this study, we show that *S. aculeata* needs to be resurrected from *S. hypnoides*, and recognized as a distinct species based on samples from Israel, Red Sea, near the type locality. *Spyridia aculeata* is characterized by the spiral arrangement of determinate branches, incompletely corticated determinate branches, naked basal segment of determinate branches, by the presence of only acropetal cortication in the nodes of determinate branches, and by uncinate spines at the terminal node and on the first and second nodes of determinate branches. In our molecular analyses based on *rbc*L and LSU rDNA, although four *Spyridia* species with uncinate spines are supported on phylogenetic trees, they are not a monophyletic group. The feature of uncinate spines in *Spyridia* is recognized as a polyphyletic character. Our phylogenetic analysis using *rbc*L and LSU rDNA sequences reveals high gene sequence divergence (6.8–7.0% for *rbc*L and 1.2–1.3% for LSU rDNA) between samples of *S. aculeata* and *S. hypnoides*. Therefore, the distribution of *S. hypnoides* may be restricted to the Indian Ocean, whereas *S. aculeata* is widely distributed in the Atlantic Ocean including the

Mediterranean Sea and the Red Sea. Another *Spyridia* species with uncinate spines, *S. alternans*, is recognized as a synonym of *S. horridula* by our detailed morphological observations of its type specimen.

KEYWORDS: LSU rDNA morphology phylogeny *rbc*L Rhodophyta *Spyridia* *S. aculeata* *S. cupressina* *S. horridula* *S. hypnoides*

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Introduction

The genus *Spyridia* was established by Harvey in 1833 based on Adriatic Sea collections of *Fucus filamentosus* Wulfen 1803 [= *S. filamentosa* (Wulfen) Harvey]. *Spyridia* is characterized by erect and filamentous thalli with numerous radially arranged lateral branches per axial cell, complete elongate cortication in primary axes, determinate lateral branches corticated only at the node, and tetrasporangia sessile on lateral branch cortical nodes. Currently, 15 *Spyridia* species are distributed worldwide in temperate to tropical seas.

Historically, 11 *Spyridia* species with uncinate spines have been recorded, *S. aculeata* (C.Agardh ex Decaisne) Kützing 1843, *S. alternans* Børgesen 1933, *S. armata* Kützing 1847, *S. berkeleyana* Montagne 1849, *S. complanata* J.Agardh 1852, *S. cupressina* Kützing 1849, *S. ericoides* (Hering) Kützing 1847, *S. hypnoides* (Bory) Papenfuss 1968*a* , *S. insignis* (J.Agardh) J.Agardh 1851, *S. horridula* F.Schmitz ex J.Agardh 1897, and *S. tetracantha* Kützing 1862. After re-examination of type material of species having uncinate spines, including *S. aculeata, S. insignis, S. ericoides*, *S. tetracantha* and *Thamnophora hypnoides* Bory in Bélinger, Papenfuss (1968*a*) synonymized these taxa as *Spyridia hypnoides* based on the nomenclatural priority of *T. hypnoides*. In 2004, John *et al*. (2004) synonymized *S. armata* under *S.*

hypnoides, and Wynne (1998) transferred *S. complanata* to *Spyridia hypnoides* subsp. *complanata* (J.Agardh) M.J.Wynne. Agardh (1851) reduced *S. berkeleyana* to variety rank as *S. aculeata* var. *berkeleyana*. Currently, four *Spyridia* species with uncinate spines on determinate lateral branches are recognized: *S. alternans*, *S. cupressina*, *S. horridula*, *S. hypnoides*. Of them, *Spyridia hypnoides* is the most widely reported species with uncinate spines in the world (Yoshida *et al*., 1990; Schneider, 2003; John *et al*., 2004; Wynne, 2017), while *S. alternans*, *S. cupressina* and *S. horridula* are reported from the Indian Ocean and have a simple nomenclatural histories (Silva *et al*., 1996).

Spyridia hypnoides was described based on a specimen from Cape Comorin, southern India (Bory de Saint-Vincent, 1834). It (including synonyms) is characterized by (1) uncinate spines near the tip of the determinate branches, (2) isometric nodal and elongated internodal cells in the main axes, (3) a radial arrangement of indeterminate and determinate branches and (4) incomplete cortication of the determinate branches (Hommersand, 1963; Papenfuss, 1968*a*). *Spyridia hypnoides* has been known for inhabiting temperate to tropical seas (Guiry & Guiry, 2020): Europe (Gómez Garreta *et al*., 2001), Atlantic Islands (John *et al*., 2004), South-east Atlantic Ocean (Lawson & John, 1987), eastern North America (Schneider & Searles, 1991), Caribbean islands (Littler & Littler, 2000), South America (Ganesan, 1990), Africa (De Clerck *et al*., 2005), Indian Ocean Islands (Silva *et al*., 1996), south-west Asia (Silva *et al*., 1996), south-east Asia (Tsutsui *et al*., 2005), and Pacific Islands (South & Skelton, 2003). Additionally, *S. aculeata* was widely recognized as a cosmopolitan taxon until Papenfuss (1968*a*) synonymized it as *S. hypnoides*.

We collected 21 worldwide samples of *Spyridia* species with uncinate spines and observed type specimens including type material of *S. alternans*. In this study, we resurrect *S. aculeata* and synonymize *S. alternans* under *S. horridula* based on morphological observation and phylogenetic relationships of *rbc*L and LSU sequence analysis.

Materials and methods

Samples

Twenty-one samples of *Spyridia* with uncinate spines were collected from South Africa, India, Israel, Brazil and Guadeloupe from 1993 to 2015 (Supplementary table S1). Of them, samples of *S. hypnoides* were collected from the type locality, and vouchers of *S. aculeata*, *S. cupressina* and *S. horridula* from near their type localities. The type specimen of *S. alternans* in New York Botanical Garden was also examined.

Morphological observations

All samples were preserved in 4–5% formalin/seawater for the morphological study and in silica gel for the molecular study. Light microscope observations were made from material stained with 1% aqueous aniline blue acidified with 0.1% diluted HCl. Photomicrographs were taken using an Olympus microscope (BX51TRF, Olympus, Tokyo, Japan) equipped with an Olympus DP71 camera. Voucher specimens used in this study were deposited both in the Seaweed Herbarium at UL Lafayette Lafayette, USA (UL) and in the herbarium of Chosun University (CUK), Gwangju, Korea. Herbarium abbreviations follow Thiers (2020).

Molecular phylogenetic analysis

DNA extraction and PCR amplification

Genomic DNA was extracted from samples preserved in silica gel (Supplementary table S1) using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA), following the manufacturer's instructions. Chloroplast-encoded *rbc*L was amplified using the primer combinations F7-R753 and F645-RrbcSstart as listed in Lin *et al.* (2001), and sequenced with the primers F7, F645, F993, R337, R753, R1150 and RrbcSstart (Freshwater & Rueness, 1994; Lin *et al.*, 2001; Gavio & Fredericq, 2002). Partial fragments of nuclear LSU rDNA were also amplified using the X, W, 28D and 28F primers (Freshwater *et al.*, 1999). Their PCR and sequencing protocols were described in Cho *et al.* (2003). Sequences were determined for both forward and reverse strands using the ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) with the ABI Prism BigDye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) at UL Lafayette.

Phylogenetic analysis

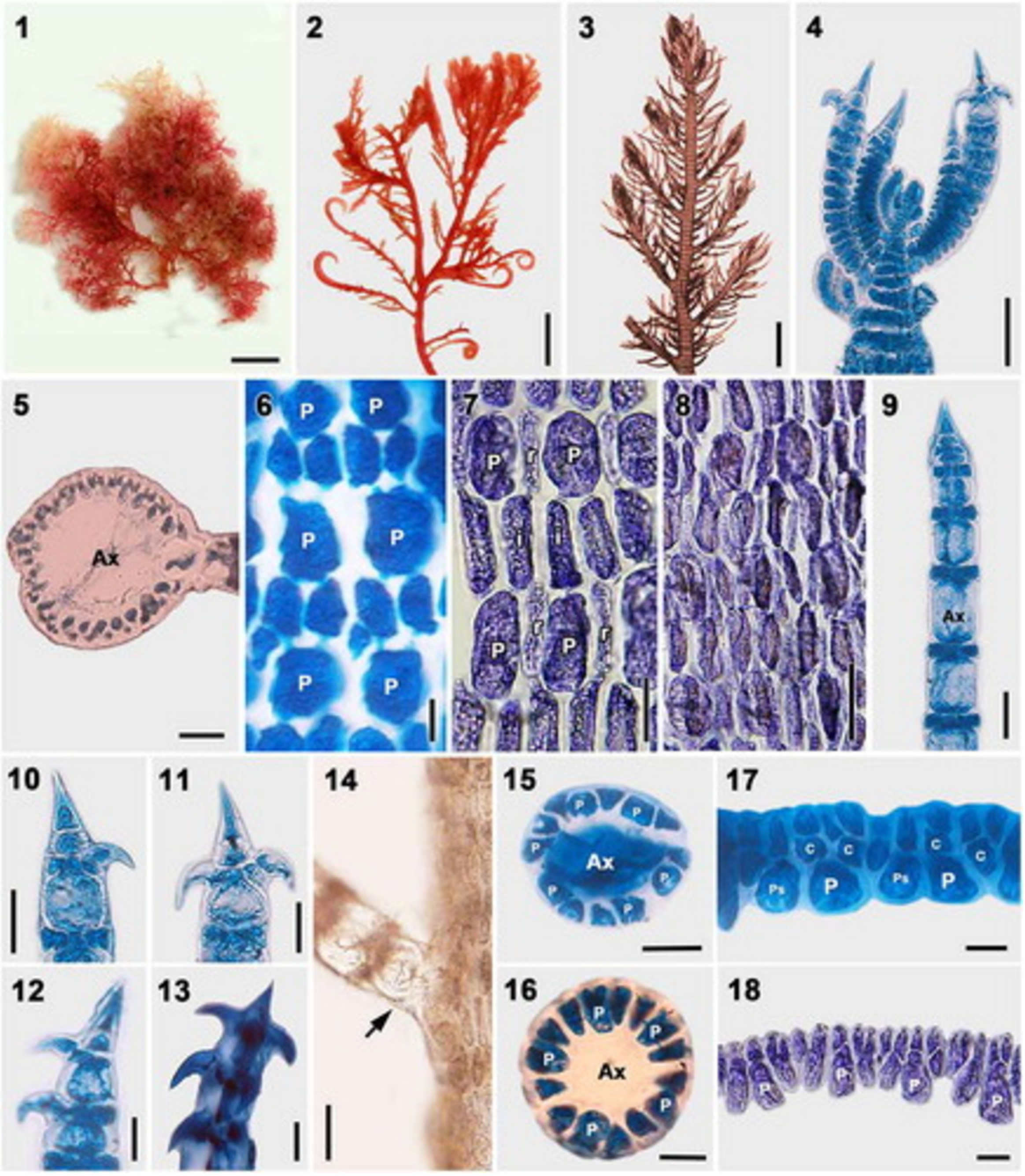
New *rbc*L and LSU rDNA sequences and other sequences obtained from GenBank were compiled (Supplementary table S1). These sequences were manually aligned by eye using the Genetic Data Environment (GDE 2.4) program (Smith *et al.*, 1994). Clustal W was used for LSU rDNA sequence alignment and trimming extraneous sequence portions. Members of the tribe Ceramieae, *Centroceras clavulatum* (C.Agardh) Montagne 1846 and *Ceramium kondoi* Yendo 1920, were selected as outgroups for phylogenetic analyses of *rbc*L and the combined dataset of *rbc*L and LSU rDNA. Maximum likelihood (ML) analyses were conducted with the RAxML HPC-AVX program (Stamatakis, 2014) implemented in the RAxML GUI 1.3.1 interface (Silvestro & Michalak, 2012) using a GTRGAMMAI model with 1,000 bootstrap replications. Bayesian inference (BI) was performed with MrBayes v. 3.2.6 software (Ronquist *et al.*, 2012) using Metropolis-coupled MCMC and the GTR + Γ + I model. We conducted two runs each with four chains (three hot and one cold) for 10 million generations, sampling trees every 1000 generations.

RESULTS

Taxonomic treatments

Spyridia aculeata (C.Agardh ex Decaisne) Kützing 1843 (Figs 1–18)

Figs 1–18. Vegetative structures of *Spyridia aculeata*. **Fig. 1**. Vegetative thallus. **Fig. 2**. Upper thallus part showing hooked indeterminate branches. **Fig. 3**. Upper thallus part showing indeterminate branches pattern. **Fig. 4**. Apex of young thallus. **Fig. 5**. Cross-section of main axis. **Fig. 6**. Cortication pattern of indeterminate branches in upper part of thallus. **Fig. 7**. Cortication pattern of indeterminate branches in middle part of thallus. **Fig. 8**. Cortication pattern of indeterminate branches in bottom part of thallus. **Figs 9–13**. Spine shapes at apex of determinate branches. **Fig. 14**. Intersection point of determinate branch on main axis lacking cortication (arrow). **Figs 15–16**. Cross-section of determinate branch. **Figs 17–18**. Cortication of determinate branch with only acropetal filament. Scale bars: Figs 1, 2 = 0.5 cm; Fig. 3 = 0.5 mm; Fig. 4 = 40 μm; Fig. 5 = 50 μm; Figs 6, 16 = 5 μm; Figs 7–9, 14 = 25 μm; Figs 10–13, 15 = 20 μm; Figs 17–18 = 10 μm



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BASIONYM: *Ceramium aculeatum* C.Agardh ex Decaisne 1841.

SYNONYMS: *Spyridia berkeleyana* Montagne 1849, *Spyridia armata* Kützing 1847.

TYPE LOCALITY: Nuweiba, Egypt.

DISTRIBUTION: Brazil, Bermuda, Egypt, Guadeloupe (F.W.I), Gulf of Mexico, Israel, Florida, North Carolina, Spain.

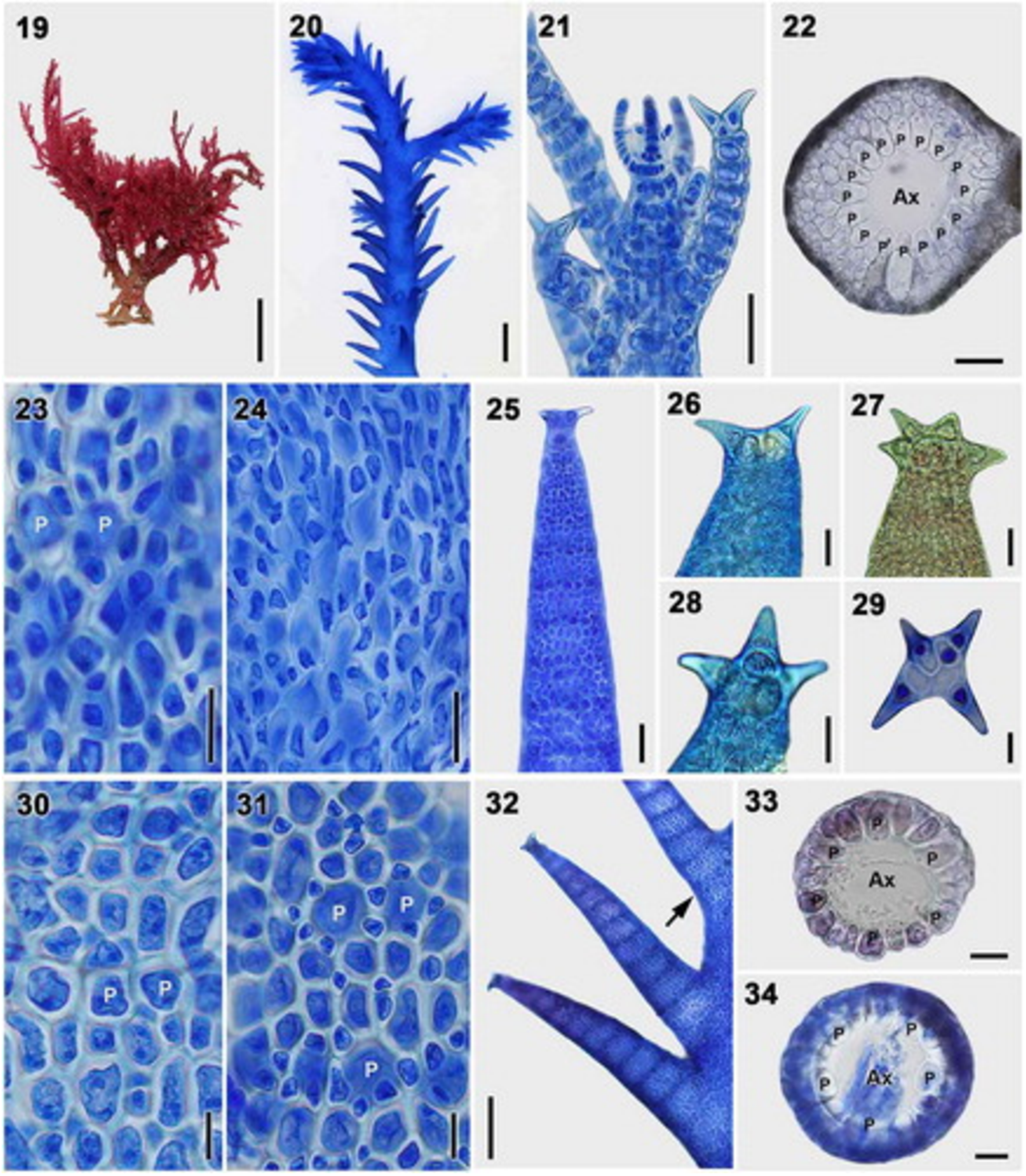
Description

Thalli are epiphytic, erect to entangled, grey-red or brownish, 2–18 cm high, and composed of main axes with indeterminate and determinate branches (Figs 1–4), sometimes with hooked apices on indeterminate branches (Fig. 2). Axial cells produce periaxial cells by alternating sequence. All periaxial cells remain at the nodes after axial cell elongation and produce cortical cells (Fig. 5). Cortication in main axes is complete and composed of periaxial cells, internodal cells and rhizoidal filaments. Internodal cells are produced in pairs from each pericentral cell, elongate reaching the next periaxial cells, connecting the periaxial cells in the segment below, and establishing secondary pit connections (Fig. 7). Rhizoidal filaments are derived primarily from internodal cells (Fig. 7). In the basal part of the thallus, rhizoidal cortical cells are cut off from both periaxial and internodal cells, and the cortication of the main axes becomes more complex with inner and outer cortical cells. Indeterminate branches are spirally arranged throughout the axes, produced from periaxial cells developed from each 6–7th segment of the main axes, and have complete cortication. Determinate branches, 377 ± 98 μm long, 59 ± 10 μm wide, are spirally arranged throughout the axes and developed from periaxial cells, terminated with spines, and have incomplete cortication (Fig. 9). Spines are simple or uncinate. One to three uncinate spines are present at the terminal node in determinate branches (Figs 9–11) with, at times, one or more lateral uncinate spines on the first and second nodes (Figs 12). The determinate branches have 11–15 cortical nodes only at the segments. Cortication in the determinate branches is 26 ± 5 μm long and composed of periaxial cells and cortical filaments. The basal segment of the determinate branches remains uncorticated (Fig. 14). Five or six periaxial cells are cut off per axial cell (Figs 15,). Each periaxial cell produces three cortical cells by slightly oblique division in an alternate sequence. The first cortical initial extends longitudinally parallel to the periaxial cells, and becomes a pseudoperiaxial cell that continues to divide in two acropetal cortical filaments (Fig. 17). The other two cortical initials are cut off obliquely from the upper ends of a periaxial cell and develop as acropetal corticating filaments (Fig. 17). Acropetal cortical filaments are 2–3 cells long and lack basipetal cortication (Fig. 18).

Reproductive thalli were not found in our collections.

Spyridia cupressina Kützing 1849 (Figs 19–34)

Figs 19–34. Vegetative structures of *Spyridia cupressina*. **Fig. 19**. Vegetative thallus. **Fig. 20**. Upper thallus part showing indeterminate branches. **Fig. 21**. Apex of young thallus. **Fig. 22**. Cross-section of the main axis. **Fig. 23**. Cortication pattern of indeterminate branches in upper part of thallus. **Fig. 24**. Cortication pattern of indeterminate branches in middle part of thallus. **Figs 25–29**. Spine shapes at apex of determinate branches. **Figs 30–31**. Cortication pattern of determinate branches. **Fig. 32**. Intersection point of determinate branch on main axis having complete cortication (arrow). **Figs 33–34**. Cross-section of determinate branch. Scale bars: Fig. 19 = 1 cm; Fig. 20 = 500 μm; Figs 21–22, 24–25 = 50 μm; Figs 23, 30–31, 33–34 = 25 μm; Figs 26–29 = 20 μm; Fig. 32 = 200 μm



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TYPE LOCALITY: Cape of Good Hope, South Africa.

DISTRIBUTION: Kenya, Madagascar, South Africa.

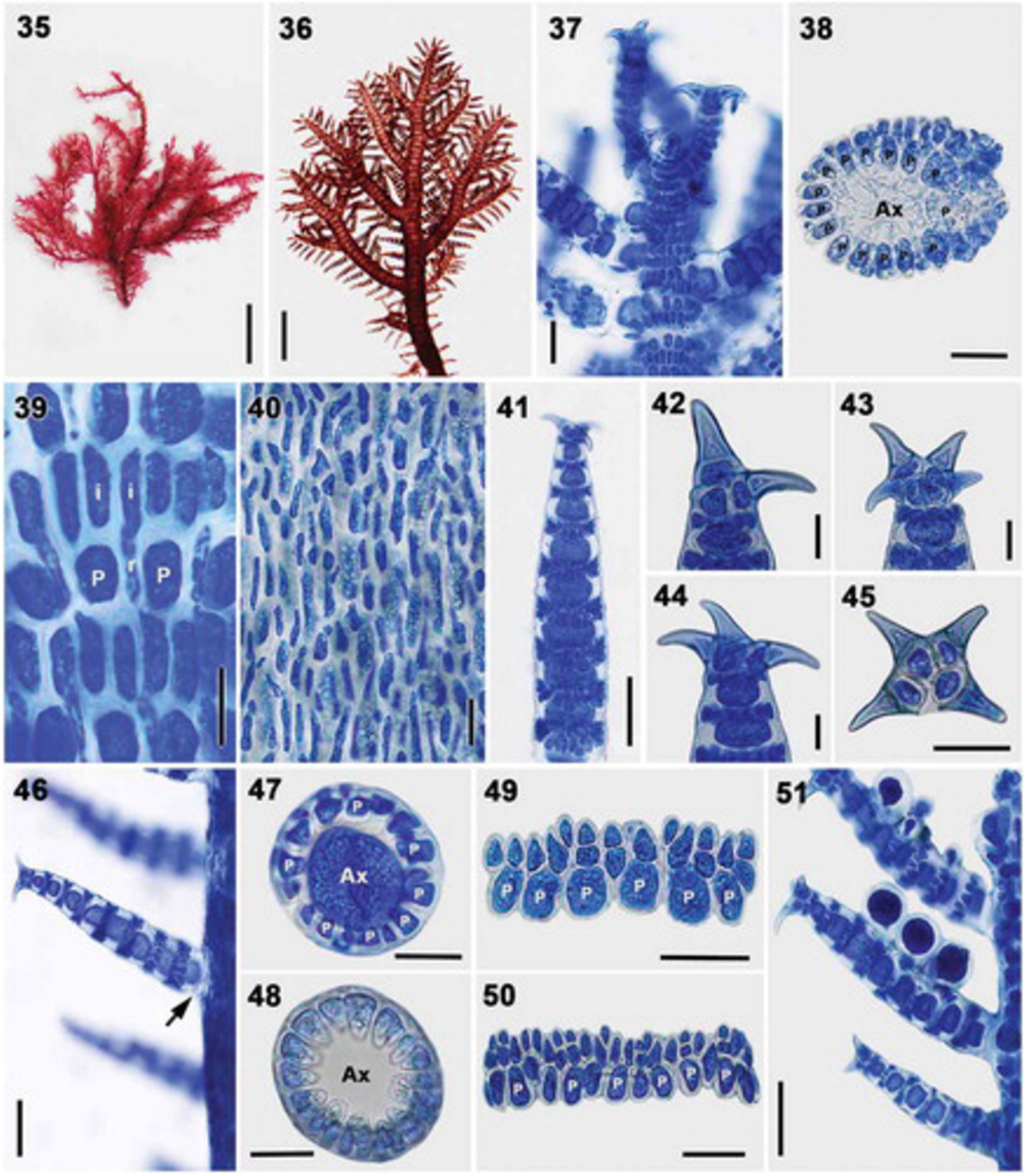
Description

Thalli are rigid, red or brownish, 2–16 cm high, irregularly branched, composed of main axes with indeterminate and determinate branches (Figs 19–21). Axial cells produce 16–17 periaxial cells in alternating sequence. All periaxial cells remain at the nodes after axial cell elongation and produce cortical cells (Fig. 22). Cortication in main axes is complete and composed of periaxial cells, internodal cells and outer cortical cells (Figs 23). Internodal cells are produced from each periaxial cell, elongate reaching the next periaxial cells, connect with the periaxial cells in the segment below, and develop outer cortical cells. Cortication of the main axes becomes rapidly more complex. Indeterminate branches are irregularly arranged throughout the axes, are produced from periaxial cells, and become completely corticated to immediately below the apices. Determinate branches, 785 ± 107 μm long, 182 ± 41 μm wide, are irregularly arranged in four rows throughout the axes (Fig. 20), develop from periaxial cells, and terminate in spines. Spines are simple or uncinate. Two to four spines are present at the terminal node (Figs 25–29). Cortication in determinate branches is complete and composed of periaxial and cortical cells (Figs 30–31). The number of segments varies, but mostly consists of 10–13 segments. The basal segments of the determinate branches are always corticated (Fig. 32). Five or six periaxial cells are cut off per axial cell (Figs 33,). Each periaxial cell produces complete cortication immediately below the apices.

Reproductive thalli were not found in our collections.

Spyridia horridula F.Schmitz ex J.Agardh 1897 (Figs 35–51)

Figs 35–51. Vegetative structures of *Spyridia horridula*. **Fig. 35**. Vegetative thallus. **Fig. 36**. Upper thallus part showing indeterminate branching pattern. **Fig. 37**. Apex of young thallus. **Fig. 38**. Cross-section of main axis. **Fig. 39**. Cortication pattern of indeterminate branches in upper part of thallus. **Fig. 40**. Cortication pattern of indeterminate branches in middle part of thallus. **Figs 41**–**45**. Spine shapes at apex of determinate branches. **Fig. 46**. Intersection point of determinate branch on main axis lacking cortication (arrow) **Figs 47**–**48**. Cross-section of determinate branch. **Figs 49**–**50**. Cortication pattern of determinate branches with acropetal filament only. **Fig. 51**. Determinate branches with tetrasporangia. Scale bars: Fig. 35 = 1 cm; Fig. 36 = 0.5 mm; Figs 37–38, 46 = 50 μm; Figs 39–40, 42–45, 47–50 = 25 μm; Figs 41, 51 = 100 μm; Fig. 32 = 200 μm



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TYPE LOCALITY: South Africa (mouth of Kowie River, Port Alfred, Cape Province).

SYNONYM: *Spyridia alternans* Børgesen 1933

DISTRIBUTION: South Africa, Pakistan.

Description

Thalli are epiphytic, erect, grey-red or brownish, mostly compressed, 6–15 cm high, and composed of main axes with indeterminate and determinate branches (Figs 35–37). Axial cells produce 14–16 periaxial cells in alternating sequence. All periaxial cells remain at the nodes after axial cell elongation and produce cortical cells (Fig.

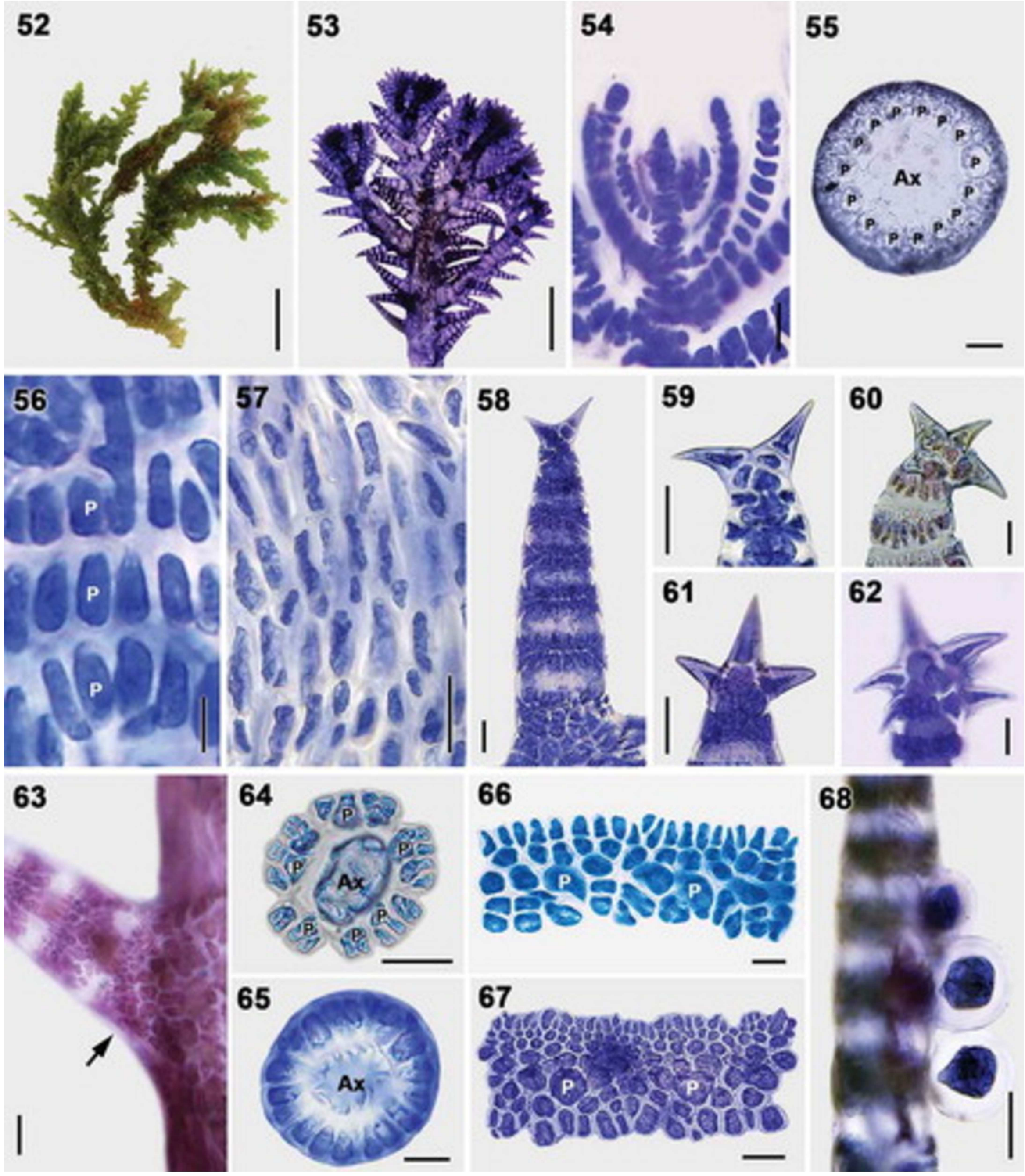
38). Cortication in main axes is complete and composed of pericaxial cells, internodal cells and rhizoidal filaments (Figs 39). The internodal cells are produced in pairs from each pericentral cell, elongate to the level of the next periaxial cells, connect with the periaxial cells in the segment below, and establish secondary pit connections (Fig. 39). Rhizoidal filaments are derived primarily from internodal cells (Fig. 39). In basal part of the thallus, rhizoidal cortical cells are cut off from both periaxial and internodal cells, and the cortication of the main axes becomes more complex. Most indeterminate branches are alternately arranged on each side of the axes, produced from a periaxial cell, and developed from each 6–9th segment of the main axes. Determinate branches are 234 ± 62 μm long, 63 ± 24 μm wide, distichously arranged on each side of the axes, developed from a periaxial cell, and terminated with 2–4 uncinate spines at the terminal node (Figs 41–45). Cortication in determinate branches composed of periaxial cells and cortical filaments, encompasses 7–9 cortical nodes, 32 ± 8 μm long, present only at the segments (Fig. 46). The basal segment of the determinate branches remains uncorticated (Fig. 46). Six or seven periaxial cells are cut off each axial cell (Figs 47). Each periaxial cell produces three cortical cells by slightly oblique division in alternate sequence (Fig. 47). The first cortical initial extends longitudinally parallel to the periaxial cells and becomes a pseudoperiaxial cell that continues to divide in two acropetal cortical filaments. The other two cortical initials are cut off obliquely from the upper ends of periaxial cell and develop as acropetal corticating filaments (Figs 49). Acropetal cortical filaments are 2–3 cells long and there is no basipetal cortication.

Tetrasporangia are tetrahedral, naked, 40 ± 12 μm wide, and produced from periaxial cells of determinate branches (Fig. 51). Spermatangial and carposporangial thalli were not found in our collections.

Spyridia hypnoides (Bory) Paperfuss 1968a (Figs 52–58)

Figs 52–68. Vegetative structures of *Spyridia hypnoides*. **Fig. 52**. Vegetative thallus.

Fig. 53. Upper thallus part showing indeterminate branching pattern. **Fig. 54**. Apex of young thallus. **Fig. 55**. Cross-section of the main axis. **Fig. 56**. Cortication pattern of indeterminate branches in upper part of thallus. **Fig. 57**. Cortication pattern of indeterminate branches in middle part of thallus. **Figs 58–62**. Spine shapes at apex of determinate branches. **Fig. 63**. Intersection point of determinate branch on main axis becoming completely corticated (arrow). **Figs 64–65**. Cross-section of determinate branch. **Figs 66–67**. Cortication pattern of determinate branches with acropetal filament and basipetal filament. **Fig. 68**. Determinate branches with tetrasporangia. Scale bars: Fig. 52 = 1 cm; Fig. 53 = 0.5 mm; Fig. 54 = 10 µm; Fig. 55 = 100 µm; Fig. 56 = 5 µm; Figs 57, 63 = 50 µm; Figs 58–62, 64–68 = 25 µm



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BASIONYM: *Thamnophora hypnoides* Bory 1834.

SYNONYMS: *Alsidium ericoides* Hering 1841, *Bindera insignis* J.Agardh 1841, *Spyridia ericoides* (Hering) Kützing 1847, *Spyridia insignis* J.Agardh 1852, *Spyridia tetracantha* Kützing 1862.

TYPE LOCALITY: Cape Comorin, Tamil Nadu, India.

DISTRIBUTION: Sri Lanka, South Africa, India.

Description

Thalli are epiphytic, erect, grey-red or brownish, bushy, 5–12 cm high, composed of main axes with indeterminate and determinate branches (Figs 52–54). Axial cells produce 14–16 periaxial cells in alternating sequence. All periaxial cells remain at the nodes after axial cell elongation and produce cortical cells (Fig. 55). Cortication in main axes is complete and composed of periaxial cells, internodal cells and rhizoidal filaments (Figs 56, 57). The internodal cells are produced in pairs from each pericentral cell, elongate to the level of the next periaxial cells, connect with the periaxial cells in the segment below, and establish secondary pit connections. Rhizoidal filaments are derived primarily from internodal cells. In basal part of the thallus, rhizoidal cortical cells are cut off from both periaxial and internodal cells, and cortication of the main axes becomes more complex. Indeterminate branches are spirally arranged throughout the axes, produced from periaxial cells in each 6–9th segment of the main axes, and completely corticated to immediately below the apices. Determinate branches are 532 ± 202 μm long, 98 ± 31 μm wide, spirally arranged throughout the axes, developed from periaxial cells, terminating in spines, and with incomplete cortication. Spines are simple or uncinate. Two to five uncinate spines are at the terminal node of determinate branches (Figs 58–62). Cortication in determinate branches, composed of periaxial cells and cortical filaments, is 75 ± 15 µm long and encompasses 8–12 cortical nodes only at the segments; however, the basal segment of the determinate branches remains corticated (Fig. 63). Five or six periaxial cells are cut off from axial cell (Figs 64). Each periaxial cell produces four or five cortical initials by slightly oblique division in alternate sequence. The first cortical initial extends parallel to the periaxial cells where it becomes a pseudoperiaxial cell that continues to divide acropetal and basipetal cortical filaments (Fig. 66). The other cortical initials develop acropetal and basipetal corticating filaments (Fig. 66). Acropetal cortical filaments are small and 4–5 cells long, and basipetal cortical filaments are large and 1–2 cells long (Fig. 67).

Tetrasporangia are tetrahedral, naked, 36 ± 2 µm wide, and produced from periaxial cells of determinate branches (Fig. 68). Spermatangial and carposporangial thalli were not found in our collections.

Molecular phylogenetic analyses

A 1,458 bp portion of the 1,467 bp *rbc*L gene (99.39% nucleotides sequenced) and partial 840 bp fragment of LSU rDNA were determined from samples of *Spyridia* species with uncinate spines for assessing their phylogenetic relationships. The interspecific divergences among *Spyridia* species with uncinate spines for *rbc*L and LSU rDNA were 4.4–7.7% and 0.2–1.4%, respectively (Table 1). The ML and Bayesian tree topologies inferred from the *rbc*L and combined dataset of *rbc*L and LSU rDNA sequences were congruent (Figs 69,70). Four *Spyridia* species with uncinate spines were supported on these phylogenetic trees. However, *Spyridia* species with uncinate spines were not resolved as monophyletic (Figs 69, 70).

Fig. 69. Phylogenetic tree based on *rbc*L sequences. Values above branches denote the Maximum likelihood bootstrap values (BS) of > 50% or the Bayesian posterior probabilities (BPP) of > 0.75. BS values of < 50% and BPP values of < 0.75 are indicated by a hyphen (-). BS values of 100 and BPP values of 1 are indicated by *

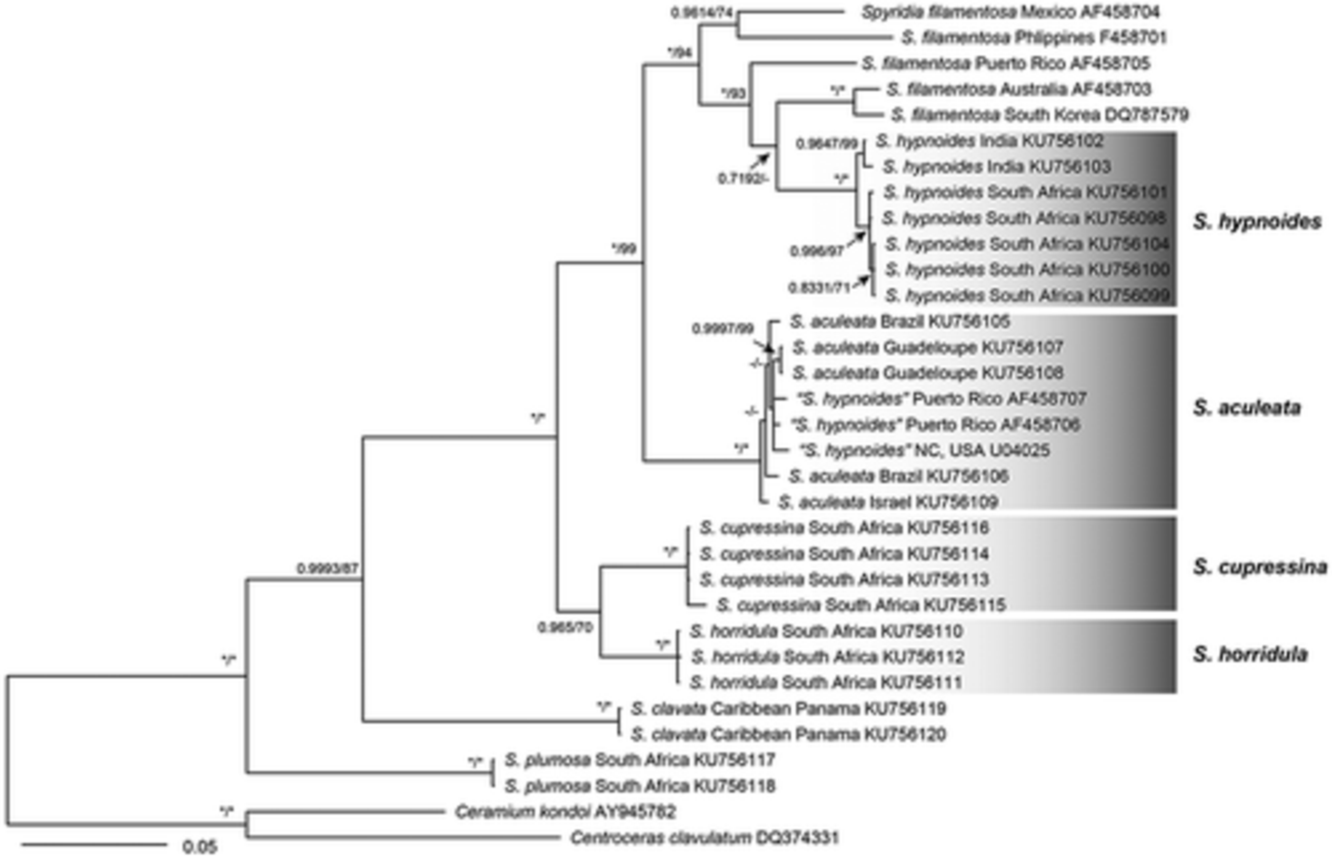

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Fig. 70. Phylogenetic tree based on the combined dataset with *rbc*L and LSU sequences. Values above branches denote the Maximum likelihood bootstrap values (BS) of > 50% or the Bayesian posterior probabilities (BPP) of > 0.75. BS values of < 50% and BPP values of < 0.75 are indicated by a hyphen (-). BS values of 100 and BPP values of 1 are indicated by *

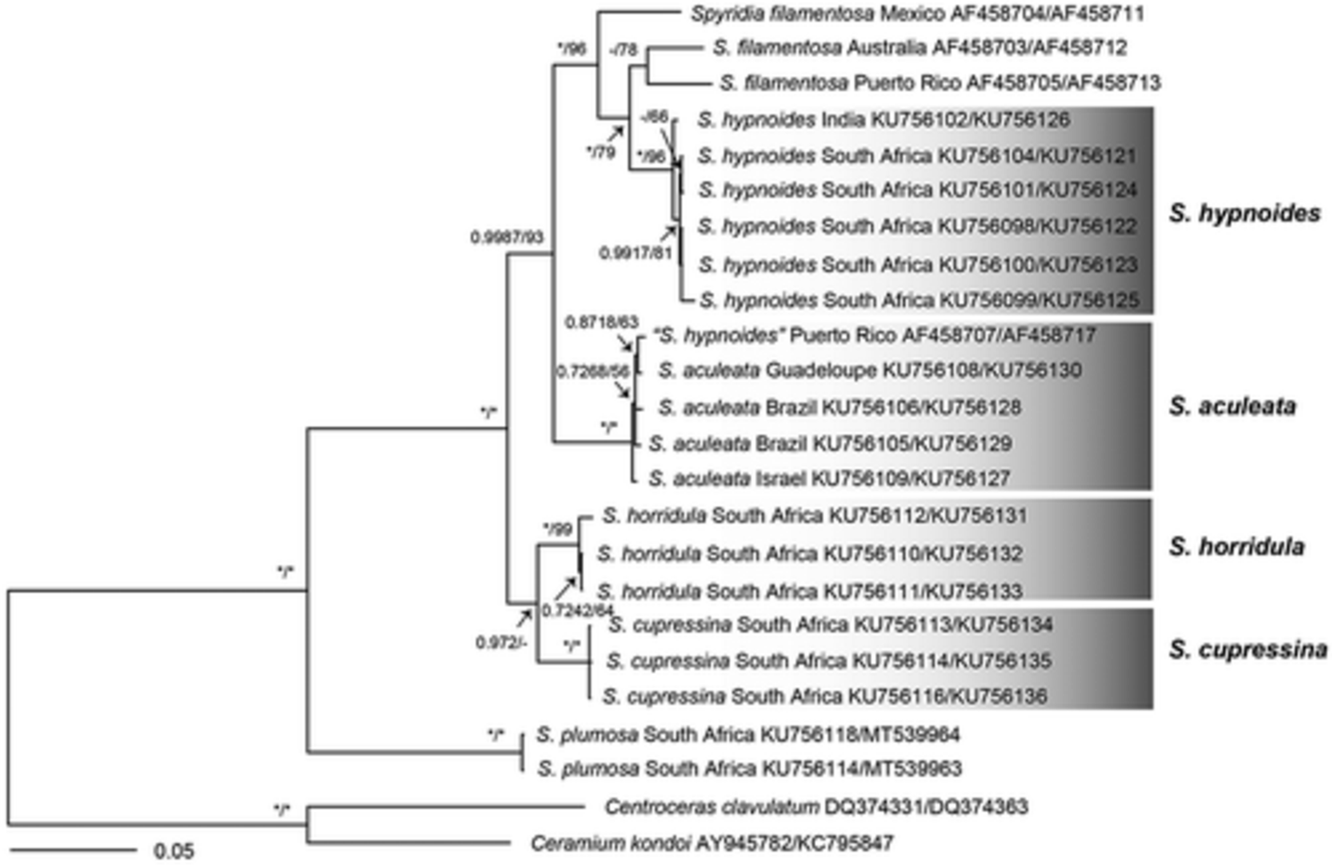

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Table 1. Comparisons (*rbc*L/LSU) of gene sequence divergence value (%) among *Spyridia* species with uncinate spines

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Key to the *Spyridia* species with uncinate spines

1. Determinate branches completely corticated so that the species name is lined up with others in the key *S. cupressina*

1. Determinate branches incompletely corticated2

2. Basal segment of the determinate branches corticated; acropetal and basipetal cortication in determinate branches *S. hypnoides*

2. Basal segment of the determinate branches naked; acropetal cortication only in determinate branches 3

3. Two-to-four uncinate spines at the terminal node of determinate branches *S. horridula*

3. One-to-three uncinate spines at the terminal node and additionally one or more lateral uncinate spines on the first and second nodes of determinate branches *S. aculeata*

DISCUSSION

Spyridia alternans, S. cupressina, S. horridula, and *S. hypnoides* are accepted in the current literature as species bearing uncinate spines in the terminal portion of their determinate branches; 11 *Spyridia* species with uncinate spines have been reported worldwide (Guiry & Guiry, 2020). Of the synonyms of *Spyridia* species with uncinate spines, *S. aculeata* originated as *Ceramium aculeatum* from Nuweiba, Egypt by Decaisne in 1841, transferred to *Spyridia* by Kützing in 1843, and considered the same species as *S. insignis* by Papenfuss (1968*b*). *Spyridia insignis* was described as *Bindera insignis* by J. Agardh in 1841, transferred to *Spyridia* by J. Agardh in 1852, and included *S. ericoides* (described as *Alsidium ericoides* Hering 1841) as a synonym (Agardh, 1852; Durairatnam, 1961). Papenfuss (1968*a*) agreed that *S. insignis* and *S. ericoides* were conspecific after examination of their type materials. Papenfuss (1968*a*) recognized these species (*S. aculeata*, *S. insignis*, *S. ericoides*) as representing a same species and suggested the combination, *Spyridia hypnoides*, based on *Thamnophora hypnoides*, the oldest conspecific taxon of *S. insignis*. However, we here recognize *S. aculeata* as a different species from the western Atlantic and the Red Sea. *Spyridia aculeata* is distinct from clades of *S. hypnoides*, *S.*

horridula and *S. cupressina* in our phylogenetic trees based on *rbc*L and combined data set of *rbc*L and LSU sequence analyses (Figs 69, 70). Although Agardh (1897) noted that *S. aculeata* was similar to *S. horridula*, it is shown to be distinct from samples of *S. horridula* by having a spiral arrangement of determinate branches, and uncinate spines at the terminal node and on the first and second nodes of determinate branches. *Spyridia aculeata* may be distinguished from the other *Spyridia* species by a spiral arrangement of determinate branches, incompletely corticated determinate branches, a naked basal segment at determinate branches, only acropetal cortication at the nodes of determinate branches, and with uncinate spines at the terminal node and on the first and second nodes of determinate branches. There is also sufficient sequence divergence between *S. aculeata* and *S. hypnoides* with regard to *rbc*L and LSU rDNA (Table 1). Therefore, in this study, we resurrect *S. aculeata* based on both morphology and molecular data.

Cortication has not been known as one of the important characters in the taxonomy of *Spyridia* because its developmental pattern was recognized as uniform. Vegetative morphological comparisons of *S. aculeata, S. cupressina, S. horridula* and *S. hypnoides* (Table 2) show that these species have complete cortication in the main axes and indeterminate branches. Main axes and indeterminate branches are completely corticated immediately below their apices by small rhizoidal cortical cells covering the internodal corticating cells. However, determinate branches have variable features in the development leading to degree and direction of the corticating filaments, features that may be used as important characters in recognizing species of *Spyridia. Spyridia aculeata*, *S. horridula* and *S. hypnoides* have incomplete cortication by having a cortical band only at the nodes of the determinate branches, whereas *S. cupressina* has complete cortication in its determinate branches. Among these three species with incomplete cortication, *S. hypnoides* has cortication in the basal node of the determinate branch, while *S. aculeata* and *S. horridula* do not. Also, in the development of cortical filaments in cortical nodes of the determinate branch, *S. aculeata* and *S. horridula* have only acropetally corticating filaments, whereas *S. hypnoides* has predominant acropetal corticating filaments and *S. cupressina* has acropetal and basipetal corticating filaments equally.

Table 2. Comparison of principal morphological features in determinate branches among *Spyridia* species with uncinate spines

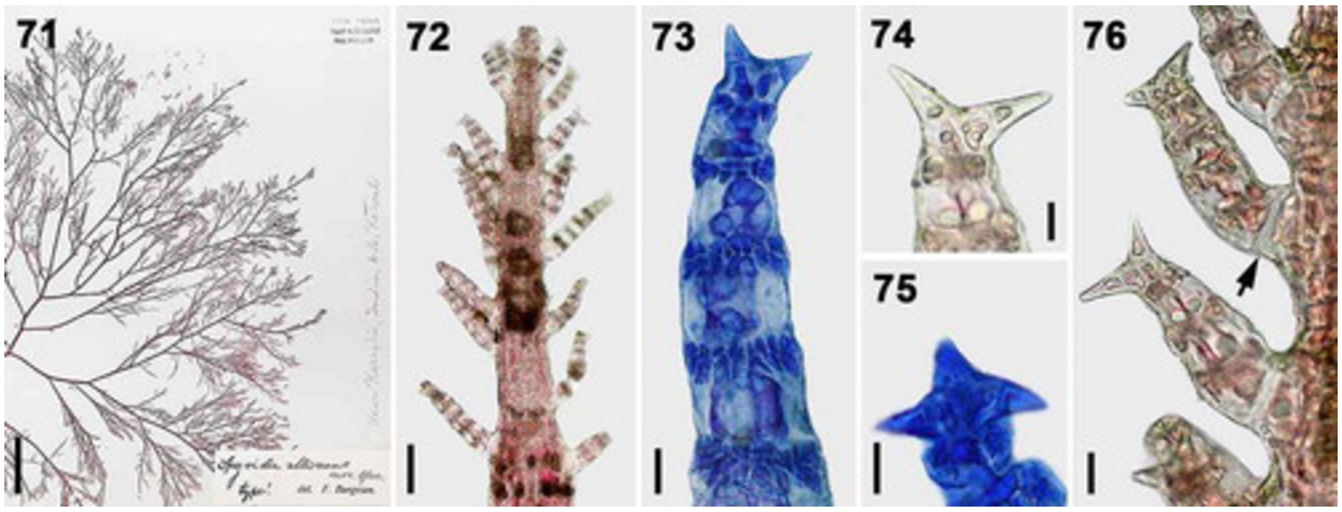
CSV Display Table

Although the length, colour, and the number of spines in *Spyridia* species were used as principal characters to define species by Kützing (1862), these features have been considered as unreliable characters in the literature. In contrast, our observations reveal morphological differences in the arrangement of spines at the nodes with *S. aculeata* having 1–2 uncinate spines each on the 1st–2nd uppermost nodes of the determinate branch and *S. hypnoides, S. horridula* and *S. cupressina* bearing 2–5 uncinate spines only on the first uppermost node of the determinate branch. Although Papenfuss (1968*a*) synonymized *S. aculeata* with *S. hypnoides* after re-examination of type materials, *S. aculeata* is distinguished from *S. hypnoides* by the arrangement of spines at the nodes. In our molecular analyses based on *rbc*L and LSU rDNA, although four *Spyridia* species with uncinate spines are supported on phylogenetic trees, they are not a monophyletic group (Figs 69, 70). The phylogeny shows that the feature of uncinate spines in *Spyridia* is a polyphyletic character.

Spyridia alternans was described from Pakistan by Børgesen in 1933 based on the alternating branching feature. In this study, we compared the type material of *S.*

alternans at the New York Botanical Garden with the other *Spyridia* species with uncinate spines based on the branching pattern and cortication of determinate branches. *Spyridia alternans* has an alternating branching pattern, incomplete cortication in determinate branches, cortical nodes with only acropetally corticating filaments in the determinate branch, the uncorticated basal node of the determinate branches, and uncinate spines only at the terminal node of the determinate branch (Figs 71–76). These features in *S. alternans* are identical to those in *S. horridula*. Therefore, although we have not succeeded in sequencing these archival materials for the molecular analysis, *S. alternans* is here recognized as a synonym of *S. horridula* based on detailed morphological observations.

Figs 71–76. Vegetative structures of *Spyridia alternans* from the New York Botanical Garden (NY #922229). **Fig. 71**. Vegetative thallus. **Fig. 72**. Apex of young thallus with determinate branches. **Fig. 73**. Cortication pattern of determinate branch. **Figs 74–75**. Spine shapes at apex of determinate branches. **Fig. 76**. Intersection point of determinate branch on main axis lacking cortication (arrow). Scale bars: Fig. 71 = 2 cm; Fig. 72 = 100 μm; Figs 73–74, 76 = 25 μm; Fig. 75 = 20 μm



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Spyridia hypnoides has been reported to have a cosmopolitan distribution from warm temperate to tropical oceans (Lawson & John, 1987; Yoshida *et al.*, 1990;

Schneider & Searles, 1991; Silva *et al*., 1996; De Clerck *et al*., 2005; Guiry & Guiry, 2020). However, morphological features and illustrations of *S. hypnoides* from the Atlantic and Mediterranean correspond to what we have observed in *S. aculeata* (Kützing, 1862: tab. 50 as *S. berkeleyana* and *S. armata*, tab. 51 as *S. aculeata*; Børgesen, 1917: fig. 228 as *S. aculeata* var. *typical*, fig. 229 as *S. aculeata* var. *disticha*, fig. 230 as *S. aculeata* f. *inermis*; Taylor, 1960: pl. 66: fig. 16, as *S. aculeata*; pl. 71: fig. 5, as *S. aculeata* var. *hypneoides*; Chapman, 1963: 179a–b, as *S. aculeata* var. *hypneoides*; Schneider & Searles, 1991: figs. 485–462). Furthermore, some *rbc*L GenBank sequences identified as *S. hypnoides* from Puerto Rico (AF458707 & AF458706) and North Carolina (U04025) are embedded within our findings for *S. aculeata*. Therefore, the distribution of *S. hypnoides* appears to be restricted to the Indian Ocean, whereas *S. aculeata* is widely distributed in the western and eastern Atlantic Ocean, the Caribbean, Mediterranean and Red Sea (Fig. 77). *Spyridia aculeata* might be widely distributed from the Atlantic to Rea Sea when water masses came from Mediterranean to Red Sea via the Proto-Gulf of Suez between 24 Ma (Chattian–Aquitanian) and 15 Ma (Late Burdigalian–Langhian) (Segev *et al*., 2017; Bialik *et al*., 2019). A population of *S. aculeata* described in 1843 from Red Sea might be segregated from theMediterranean population by 8 Ma (Tortonian) before the opening of the Suez Canal in 1869.

Fig. 77. Distributions of *Spyridia aculeata* (● : localities of samples collected from this study, ○ : localities of samples corresponding morphological features or illustrations of species in literatures and DNA sequences in GenBank) and *S. hypnoides* (■ : localities of samples collected from this study, □ : localities of samples corresponding morphological features or illustrations of species in literatures and DNA sequences in GenBank)

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Although *S. horridula* has been known as an endemic species of South Africa (Silva *et al.*, 1996), its distribution is expanded to Pakistan in this study. *Spyridia cupressina* has been known from the South-west Indian Ocean including South Africa, Kenya and Madagascar (Silva *et al.*, 1996).

Supplemental material

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary Information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at https://doi.org/10.1080/09670262.2020.1840634.

Supplementary table S1. List of specimen information used in molecular analyses. Additional *rbc*L and LSU sequences downloaded from GenBank are provided.

Author contributions

B.Y. Won: original concept, experiment, drafting and editing the manuscript; S. Fredericq: original concept, funding and editing the manuscript; T.O. Cho: funding and editing the manuscript.

Additional information

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