UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

NATALIA LEOCADIO ROSSIGNOLO

Revisão do gênero *Sirodotia* (Batrachospermales, Rhodophyta)

São José do Rio Preto

2020

NATALIA LEOCADIO ROSSIGNOLO

Revisão do gênero *Sirodotia* (Batrachospermales, Rhodophyta)

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Microbiologia, junto ao Programa de Pós-Graduação em Microbiologia, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

Financiadora: FAPESP – Proc. 2016/16320-2

Orientador: Prof. Dr. Orlando Necchi Junior

São José do Rio Preto

2020

R834r

Rossignolo, Natalia Leocadio

Revisão do gênero Sirodotia (Batrachospermales, Rhodophyta) / Natalia Leocadio Rossignolo. -- São José do Rio Preto, 2020

121 f. : il., tabs.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto

Orientador: Orlando Necchi Junior

1. Alga vermelha. 2. Freshwater algae. 3. Filogenia. 4. Molecular Biology. 5. Morfologia. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

NATALIA LEOCADIO ROSSIGNOLO

Revisão do gênero *Sirodotia* (Batrachospermales, Rhodophyta)

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Mircrobiologia, junto ao Programa de Pós-Graduação em Microbiologia, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

Financiadora: FAPESP – Proc. 2016/16320-2

Comissão Examinadora

Prof. Dr. Orlando Necchi Junior
UNESP – Câmpus de São José do Rio Preto
Orientador

Profa. Dra. Mariana Cabral de Oliveira
USP – São Paulo

Profa. Dra. Mutue Toyota Fujii
Instituto de Botânica – São Paulo

Profa. Dra. Valéria Cassano
USP – São Paulo

Profa. Dra. Inessa Lacativa Bagatini
UFSCAR – São Carlos

São José do Rio Preto
28 de agosto de 2020.

“Aos meus amados pais, marido André Luiz e filho João Antonio,
por toda paciência, suporte e amor dedicados a min e que
sempre acreditaram que eu seria capaz de atingir meus
objetivos e alcançar meus sonhos”.

AGRADECIMENTOS

Ao Prof. Dr. Orlando Necchi Jr., minha sincera gratidão por seus ensinamentos, pela confiança em mim depositada, pela compreensão, paciência, incentivo e orientação, para que pudéssemos concluir este trabalho.

Aos meus pais pelo alicerce, incentivo, paciência, carinho, e, principalmente, por estarem presentes em todos os momentos da minha vida.

Ao meu marido e filho por toda compreensão, paciência e amor dedicados a min, e principalmente por estarem sempre ao meu lado me apoiando e incentivando.

Aos meus sogros, Bernadete e Aston, por toda ajuda, apoio e incentivo durante esses anos.

Ao Ibilce, Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista do campus de São José do Rio Preto, São Paulo, pelo espaço fornecido.

À coordenação do Programa de Pós-Graduação em Microbiologia e aos funcionários da Seção de Pós-graduaçao, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista do campus de São José do Rio Preto, São Paulo, pelo incentivo e encaminhamento dos expedientes nas várias etapas do Doutorado.

Agradeço à fundação de Amparo e Pesquisa do Estado de São Paulo (FAPESP) pela bolsa de Doutorado (Proc. 2016/16320-2) e auxílio à pesquisa ao orientador (Proc. 2016/07808-1).

Aos colegas do Laboratório B.E.T.A. (Biologia, Ecologia e Taxonomia de Algas) pelo excelente convívio durante esse tempo.

“Aprender é a única coisa de que a mente nunca se cansa, nunca tem medo e nunca se arrepende”.

Leonardo da Vinci (Pensador, 2020)

RESUMO

Gêneros da ordem das algas vermelhas de águas continentais, Batrachospermales, foram recentemente investigados usando evidências moleculares e morfológicas, mas o gênero *Sirodotia* ainda não havia sido revisado. O presente estudo teve como objetivos: inferir as relações filogenéticas das espécies dentro do gênero *Sirodotia* e com outros gêneros da ordem Batrachospermales; conhecer os limites de variação intra e interespecífica com base na análise de três marcadores moleculares: gene plastidial que codifica a subunidade grande da RuBisCO (*rbc*L), região de "barcode" do gene mitocondrial que codifica a subunidade 1 da citocromo *c* oxidase (COI-5P) e região de "barcode" do gene nuclear que codifica a subunidade grande do ribossomo (rDNA LSU); e avaliar os caracteres morfológicos de valor diagnóstico para a delimitação de espécies do gênero em âmbito mundial à luz dos novos dados moleculares. Foram analisadas 40 amostras de *Sirodotia* provenientes de várias regiões do mundo, incluindo África, Ásia, Australásia, América do Norte, América do Sul e Europa, além de espécimes-tipo de seis espécies. Análises filogenéticas concatenadas e de um único gene (*rbc*L, COI-5P e LSU) mostraram o gênero *Sirodotia* como grupo monofilético com alto suporte, evidenciando clados ou grupos bem definidos, com nítida repartição biogeográfica delimitando as espécies, além de alta divergência interespecífica (2,1-8,1% e 4,4-10,5%) e baixa variação intraespecífica (0-2,4% e 0-3,8%) para *rbc*L e COI-5P, respectivamente. As análises das sequências de LSU revelaram valores de divergência interespecíficas menores (0,7-3,3%) do que as sequências de COI-5P indicando menor poder de resolução como "barcode". Os caracteres morfológicos diagnósticos previamente utilizados para distinguir o gênero (carpogônio assimétrico com protuberância basal e carposporófito difuso com filamentos prostrados produzindo ramos eretos com carposporângios) foram confirmados neste estudo. Algumas espécies apresentaram padrão biogeográfico bem definido: *S. delicatuliformis* ocorrência no Brasil e Costa Rica, *S. amazonica* (regiões centro-oeste e norte do Brasil) e *S. cryptica* (centro-oeste do Brasil), *S. assamica* (nordeste da Índia), *S. aquiloamericana* (sul da América do Norte), *S. huillensis* (sul e sudeste da África) e *S. kennedyi* (centro-sul da África). Assim, distribuição geográfica pode ser usada como critério adicional para o reconhecimento dessas espécies. Os seguintes caracteres foram considerados diagnósticos para distinguir as espécies do gênero: número de células dos fascículos primários, arranjo

dos espermatângios, parte do carpogônio que produz os filamentos gonimoblásticos e dimensões do carposporângio. Com base na combinação de dados moleculares, morfológicos e biogeográficos foram reconhecidas nove espécies, cinco já descritas (*S. assamica*, *S. delicatula*, *S. huillensis, S. kennedyi* e *S. suecica*) e quatro novas espécies propostas neste trabalho (*S. amazonica*, *S. aquiloamericana*, *S. cryptica* e *S. delicatuliformis*). Das espécies previamente consideradas taxonomicamente aceitas no gênero, três (*S. cirrhosa*, *S. gardneri* e *S. huangshanensis*) não puderam ser reavaliadas, pois os espécimes tipo não estavam disponíveis para análise e os caracteres utilizados não foram considerados taxonomicamente informativos; e quatro espécies foram sinonimizadas neste trabalho (*S. segawae*, *S. sinica* e *S. yutakae* com *S. suecica;* e *S. ateleia* com *S. delicatula*).

Palavras–chave: Batrachospermales, COI-5P, LSU, *rbc*L, *Sirodotia*, morfologia, sistemática molecular.

ABSTRACT

Genera of the freshwater red algal order Batrachospermales were recently investigated using molecular and morphological evidences, but the genus *Sirodotia* had not yet been revised. The goals of this study were: to infer the phylogenetic relationships of the species within the genus *Sirodotia* and with other genera of the order Batrachospermales; to ascertain the limits of intra and interspecific variation, based on the analysis of three molecular markers: the plastid gene that encodes the RuBisCO large subunit (*rbc*L), the barcode region of the mitochondrial gene that encodes cytochrome *c* oxidase subunit 1 (COI-5P) and the barcode region of the nuclear gene that encodes the large ribosome subunit (rDNA LSU); and to evaluate the morphological characters of diagnostic value for delimitation of species in the genus worldwide in the light of the new molecular data. Forty samples of *Sirodotia* from different regions of the world were analyzed, including Africa, Asia, Australasia, North America, South America and Europe, in addition to type specimens of six species. Concatenated and single gene analyses showed the genus *Sirodotia* as a monophyletic group with high support, showing well-defined clades or groups, with clear biogeographic distribution delimiting species, in addition to high interspecific divergence (2.1-7% and 4.4-10.5%) and low intraspecific variation (0-2.4% and 0-3.8%) for *rbc*L and COI-5P, respectively. The analysis of the LSU sequences revealed lower interspecific divergence values (0.7-3.3%) than the COI-5P sequences (4.4-10.5%) indicating lower resolution as a barcode marker. The diagnostic morphological characters previously used to distinguish the genus (asymmetric carpogonium with basal protuberance and diffuse carposporophyte with prostrate filaments producing erect branches with carposporangia) were confirmed in this study. Some species showed a well-defined biogeographic pattern: *S. delicatuliformis* occurring in Brazil and Costa Rica, *S. amazonica* (midwestern and northern Brazil), *S. cryptica* (midwestern Brazil), *S. assamica* (northeastern India), *S. aquiloamericana* (southern North America), *S. huillensis* (southern and southeastern Africa) and *S. kennedyi* (south-central Africa). Thus, geographic distribution can be used as an additional criterion for the recognition of these species. The following characters were considered diagnostic to distinguish species in the genus: number of cells of the primary fascicles, arrangement of spermatangia, part of the carpogonium that produces the gonimoblastic filaments and dimensions of the carposporangia. On the basis of a combination of molecular, morphological and biogeographic data nine species were

recognized, five already described (*S. assamica*, *S. delicatula*, *S. huillensis*, *S. kennedyi* and *S. suecica*) and four new species proposed in this work (*S. amazonica*, *S. aquiloamericana*, *S. cryptica* and *S delicatuliformis*). Among the remaining species taxonomically accepted in the genus, three (*S. cirrhosa*, *S. gardneri* and *S. huangshanensis*) could not be re-evaluated, because the type specimens were not available for analysis and the characters used were not considered taxonomically informative; and four species were synonymyzed in this study (*S. segawae*, *S. sinica* and *S. yutakae* with *S. suecica*; and *S. ateleia* with *S. delicatula*).

Keywords: Batrachospermales, COI-5P, LSU, molecular systematics, morphology, *rbc*L, *Sirodotia*.

LISTA DE ILUSTRAÇÕES

Figura 1 – Filogenia das algas vermelhas segundo autores recentes (Muñoz-Gomes et al., 2017) com a proposição de um novo Subfilo, Proteorhodophytina . 16

Figura 2 – Maximum likelihood phylogenetic tree based on *rbc*L DNA sequences. 35

Figura 3 – Distance tree (neighbor joining) of COI-5P DNA sequences for *Sirodotia* species and Batrachospermales outgroup taxa. 36

Figura 4 – *Sirodotia assamica sp. nov.* 39

Figura 5 – Phylogenetic tree (ML) showing the relationship of species within *Sirodotia* and genera of the Batrachospermales and Thoreales based on *rbc*L, COI-5P and LSU concatenated sequence data. 63

Figuras 6-10 – *Sirodotia amazonica* (photomicrographs of the holotype, SJRP 31924). 77

Figuras 11-15 – *Sirodotia aquiloamericana* (photomicrographs of the holotype, BHO-0437). 77

Figuras 16-21 – *Sirodotia assamica* (photomicrographs of SJRP 32583, SJRP 32584). 78

Figuras 22-26 – *Sirodotia cryptica* (photomicrographs of the holotype, samples SJRP 32575). 78

Figuras 27-31 – *Sirodotia delicatula* (photomicrographs of BHO A-0984). 79

Figuras 32-38 – *Sirodotia delicatuliformis* (photomicrographs of holotype, SJRP 31918). 79

Figuras 39-44 – *Sirodotia huillensis* (photomicrographs of BHO A-1447). 80

Figuras 45-47 – *Sirodotia kennedyi* (photomicrographs of BHO A-0946). 80

Figuras 48-52 – *Sirodotia suecica* (photomicrographs of BHO A-0266, SJRP32582, SJRP32583). 80

Fig. S1 – Maximum likelihood phylogenetic tree based on *rbc*L. 111

Fig. S2 – Distance tree (neighbor-joining) based on COI-5P sequences. 112

Fig. S3 – Graphical representation of the results of Automatic Barcode Gap Discovery (ABGD) for the COI-5P sequences of the *Sirodotia* genus used in this study. 113

Fig. S4 – Distance tree (neighbor-joining) based on LSU sequences. 114

LISTA DE TABELAS

Tabela 1 – Gêneros da ordem Batrachospermales, família Batrachospermaceae. 19

Tabela 2 – Description of collecting sites and GenBank accession numbers for samples of *Sirodotia assamica* sp. nov. from India analyzed in this study. 33

Tabela 3 – Comparative morphology and distribution of *Sirodotia* species. 40

Tabela 4 – Relação das espécies *Sirodotia* anteriormente consideradas taxonomicamente aceitas e as reconhecidas neste estudo. Os nomes em negrito indicam as nove espécies reconhecidas neste estudo. 116

Table S1 – Sequences of *rbc*L for *Sirodotia* species from Genbank used in the analysis. 46

Table S2 – Sequences of COI-5P for *Sirodotia* species obtained from Genbank used in the analyses. 48

Table S3 – Morphological characters of *Sirodotia assamica* sp. nov. specimens examined in this study. 49

Table S4 – Reproductive characters of *Sirodotia assamica* sp. nov. specimens examined in this study. 50

Table S5 – Species, collection information, and GenBank accession numbers for specimens of *Sirodotia* used in this study. 94

Table S6 – Sequences of *rbc*L, COI-5P and LSU *Sirodotia* species obtained from Genbank used in the analyzes. 98

Table S7 – Outgroup sequences used in *rbc*L, COI and LSU concatenated alignment for phylogenetic analyses. 99

Table S8 –Morphological characters of *Sirodotia* specimens examined in this study or reported in relevant works. 101

Table S9 – Reproductive characters of *Sirodotia* specimens examined in this study or reported in relevant works. 104

Table S10 – Comparative morphological characters and geographic distribution of some species of *Sirodotia*. 108

APRESENTAÇÃO DA TESE

O presente trabalho consiste em uma revisão mundial do gênero *Sirodotia* (Rhodophyta, Batrachospermales). Está estruturado em dois capítulos, precedidos por uma introdução geral e seguidos por conclusões gerais. Os dois capítulos são referentes a dois artigos: o primeiro capitulo representa a primeira parte do trabalho (artigo publicado) com a descrição de uma nova espécie de *Sirodotia* para o continente Asiático (Índia); o segundo capitulo corresponde ao trabalho geral deste projeto, referente a revisão mundial de *Sirodotia* contendo as análises dos três marcadores utilizados, parte taxonômica com chave de identificação, descrição das espécies reconhecidas, discussão e conclusões referentes aos resultados gerais obtidos.

SUMÁRIO

1. INTRODUÇÃO GERAL 15

1.1. O gênero *Sirodotia* 20

1.2. Hipóteses e objetivos gerais do estudo 21

1.2.1. Hipotéses 21

1.2.2. Objetivos 22

1.3. Referências 23

2. CAPITULO 1: **Molecular and morphological evidence for a new species in the genus *Sirodotia* (Batrachospermales, Rhodophyta) from the State of Assam, India** 28

2.1. Abstract 29

2.2. Introduction 30

2.3. Material and Methods 32

2.4. Results And Discussion 34

2.4.1. Molecular analyses 34

2.4.2. Morphological analyses 37

2.5. Conclusion 41

2.6. References 41

2.7. Supporting Information 45

3. CAPITULO 2: Revision of the genus *Sirodotia* (Batrachospermales, Rhodophyta) with description of four new species 51

3.1. Abstract 52

3.2. Introduction 53

3.3. Material and Methods 56

3.3.1. Morphological analyses 56

3.3.2.Molecular analyses 58

3.4. Results 60

3.4.1. Molecular analyses 60

3.4.2. Morphological analyses 63

3.4.2.1 Taxonomic Treatment 64

3.4.2.2. Species description 66

3.4.2.3. Doubtful species 81

3.5. Discussion 81

3.6. References 85

3.7. Supporting Information 92

4. CONCLUSÕES GERAIS 115

REFERÊNCIAS 119

1. INTRODUÇÃO GERAL

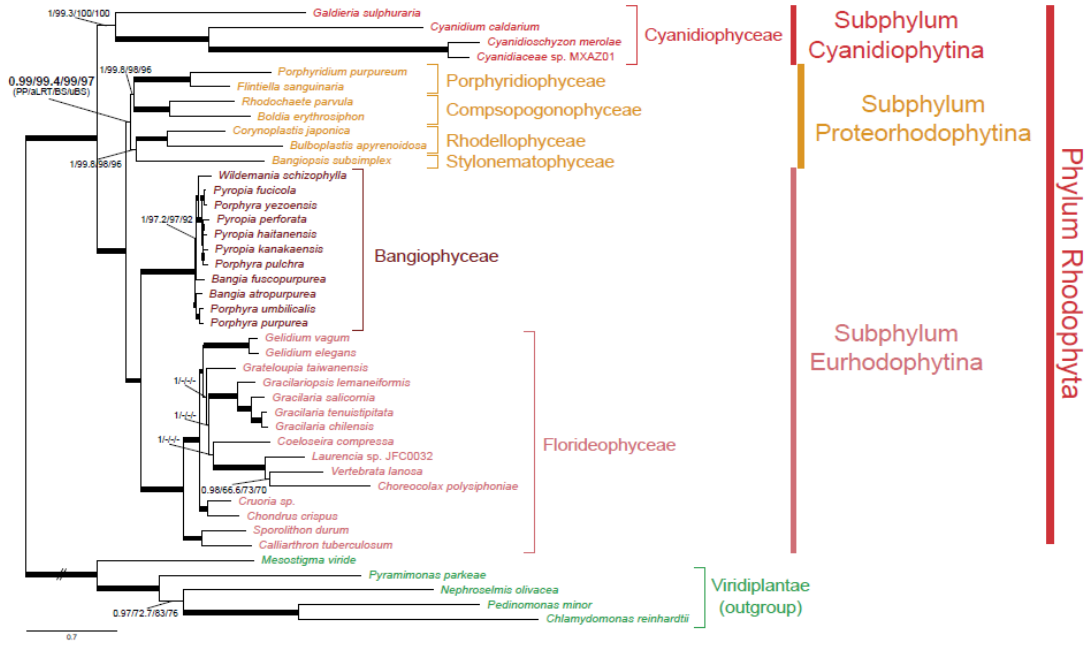
O filo Rhodophyta representa umas das linhagens mais antigas de algas e é considerado um grupo irmão das algas verdes e das glaucófitas, todos com cloroplastos derivados de endosssimbiose primária (LOPEZ-BAUTISTA, 2010; DE CLERCK *et al*., 2012). As rodófitas são, em sua maioria, macroscópicas, fotossintetizantes e habitantes de ambientes marinhos (98%), mas muitos táxons ocorrem exclusivamente em hábitats de água doce e nascentes de água quente e ácida. Algumas rodófitas também foram relatadas em florestas tropicais como membros de comunidades subaéreas (YOON *et al*., 2004; GURGEL & LOPEZ-BAUTISTA, 2007). Em regiões tropicais e subtropicais dos ecossistemas marinhos costeiros, Rhodophyta representa mais de 60% das macroalgas (NORTON *et al*., 1996). As rodófitas continentais ocorrem geralmente em águas correntes e estão amplamente distribuídas em bacias de drenagem de regiões árticas a tropicais (NECCHI *et al*., 1999). No Brasil foram registradas 39 espécies de rodófitas continentais (NECCHI, 2017; dados não publicados).

As algas vermelhas representam um distinto grupo de organismos que possuem apenas clorofila a, contêm ficobiliproteínas (aloficocianina, ficocianina e ficoeritrina) responsáveis pela coloração geralmente avermelhada destas algas, que ocorrem na forma de ficobilissomos em tilacoides não empilhados. Os membros desse grupo não apresentam flagelos e centríolos em nenhum estágio de vida (GABRIELSON *et al*., 1990; GRAHAM & WILCOX, 2009). O cloroplasto é envolto por duas membranas e produz amido das florídeas (material de reserva), que é armazenado no citoplasma. Atualmente é amplamente aceito que o cloroplasto das algas vermelhas se originou através da endossimbiose primária de uma cianobactéria, sendo que essa organela compartilha um ancestral comum com as algas verdes e glaucófitas (BHATTACHARYA *et al*., 2004; RODRIGUEZ-EZPELETA *et al*., 2005).

O sistema de classificação do filo Rhodophyta tem passado por modificações importantes com base em estudos moleculares e ultraestruturais que visam melhor representar as relações filogenéticas (SAUNDERS & HOMMERSAND, 2004; YOON *et al.* 2006; QIU *et al*., 2016). Até o fim dos anos noventa, o filo Rhodophyta era dividido em duas classes ou subclasses, Florideophyceae (Florideophycideae) e Bangiophyceae (Bangiophycideae), baseadas nas características morfológicas e histórico de vida (SHEATH, 1984; GABRIELSON *et al*., 1985; GARBARY &

GABRIELSON, 1990). Estudos moleculares têm se baseado amplamente em alguns genes plastidiais e nucleares focados em uma ampla diversidade de linhagens dentro das Florideophyceae. Uma das primeiras consequências dessas análises foi a separação do filo Rhodophyta em dois subfilos, Cyanidiophytina com uma única classe Cyanidiophyceae, e o subfilo Rhodophytina composto por seis classes: Bangiophyceae, Compsopogonophyceae, Florideophyceae, Porphyridiophyceae, Rhodelophyceae e Stylonematophyceae (YOON *et al.*, 2006). As classes Bangiophyceae e Florideophyceae formaram um grupo monofilético, mas as relações entre as classes restantes permaneceram controversas (MULLER *et al*., 2001; YOON *et al*., 2006, 2010; YANG *et al*., 2010; SCOTT *et al*., 2011). Em estudo recente, Muñoz-Gomes *et al.* (2017) aplicaram análise filogenômica a um rico conjunto de dados de genomas plastidiais e propuseram um novo subfilo Proteorhodophytina composto por quatro classes: Porphyridiophyceae, Compsopogonophyceae, Rhodellophyceae e Stylonematophyceae. Algas destes grupos apresentaram os maiores genomas plastídiais já sequenciados até o presente.

Figura 1. Filogenia das algas vermelhas segundo autores recentes (*Muñoz-Gomes et al.,* 2017) com a proposição de um novo Subfilo, Proteorhodophytina.



Fonte: Muñoz-Gomes *et al*., 2017.

No outro subfilo (Eurhodophytina) encontra-se a classe Florideophyceae que forma uma linhagem monofilética contendo aproximadamente 7.000 espécies descritas (GUIRY, 2012) que exibem uma grande variedade de estruturas

morfológicas e ultraestruturais e possuem estratégias reprodutivas complexas. Acredita-se que o número de espécies possa chegar a 20.000 devido às recentes descobertas de espécies crípticas (GRAHAM & WILCOX, 2009). Elas representam cerca de 95% dos táxons conhecidos de algas vermelhas e são importantes fontes de alimentos (por exemplo, nori) e produtos industriais (por exemplo, ágar e carragenana) (QIU *et al*., 2015). O número de ordens reconhecidas varia de 16 (YOON *et al*., 2006) a 23 (LE GALL & SAUNDERS, 2007) e tende a crescer com a realização de estudos de grupos específicos baseados em dados moleculares.

As ordens e famílias de Florideophyceae foram classificadas, em grande parte, com base no tipo de histórico de vida e o modo de reprodução sexuada. Membros desta classe crescem por meio de células apicais e consistem de filamentos ramificados nos quais as células estão ligadas através de “pit-connections” (conexões intercelulares entre células adjacentes), as quais, em nível estrutural, formam os chamados “pit-plugs” (GRAHAM & WILCOX, 2009). As características ultraestruturais dos “pit-plugs”, como número e arranjo das camadas, são importantes para delimitação das ordens. Vários tipos de “pit-plugs” foram identificados por diferirem no número de “cap layers” (zero, um ou dois) e pela presença ou ausência de uma “cap membrane”. Nove ordens (Hildenbrandiales, Corallinales, Acrochaetiales, Palmariales, Batrachospermales, Ahnfeltiales, Bonnemaisoniales, Gracilariales e Rhodogorgonales) foram estabelecidas principalmente pelas características ultraestruturais dos “pit-plugs” (PUESCHEL & COLE, 1992; FRESHWATER *et al*., 1994; LEE, 2008; ELORANTA *et al*., 2011).

Dentro da classe Florideophyceae, na subclasse Nemaliophycidae, encontram-se as rodófitas exclusivamente de águas continentais que pertencem às ordens Balbaniales, Batrachospermales e Thoreales (LAM *et al*., 2015). A maior parte da diversidade de espécies de algas vermelhas de água doce encontra-se na ordem Batrachospermales com aproximadamente 200 espécies em 19 gêneros (ENTWISLE *et al*., 2009; GUIRY & GUIRY, 2020). Batrachospermales é distinta das demais ordens de Nemaliophycidae com base na seguinte combinação de caracteres: (i) talos heterotríqueos, uniaxiais, gelatinosos ou cartilaginosos; (ii) células axiais com filamentos assimiladores laterais de crescimento determinado; (iii) “pit-plug” com duas camadas, com a camada externa expandida e abaulada; (iv) ausência de tetrasporângio e ocorrência de meiose somática dando origem a eixos haplóides; e (v) múltiplos cloroplastos discóides sem pirenoides; (PUESCHEL & COLE, 1982;

GARBARY & GABRIELSON, 1990; KUMANO, 2002; ENTWISLE *et al*., 2009; PAIANO *et al*., 2018).

A ordem Batrachospermales era tradicionalmente composta por três famílias: Batrachospermaceae, Lemaneaceae e Psilosiphonaceae (SAUNDERS & HOMMERSAND, 2004). Batrachospermales foi foco de revisão por Entwisle *et al.* (2009), o qual representa o esquema taxonômico mais recente e amplo para a ordem. As principais novidades, além do reconhecimento de uma única família (Batrachospermaceae com nove gêneros) foram: reconhecimento provisório do gênero *Batrachospermum*, visto que tem sido demonstrado como parafilético em todas análises filogenéticas moleculares; proposição do novo gênero *Kumanoa*, resultado da elevação das antigas seções *Contorta e Hybrida* do gênero *Batrachospermum*; e o reconhecimento de oito seções no gênero *Batrachospermum* – *Acarposporophytum* (rebaixado do nível subgênero para seção), *Aristata*, *Batrachospermum*, *Macrospora* (separada de *Aristata*), *Setacea, Turfosa* e *Virescentia.* Para a maioria das seções havia grande carência de análises representativas incluindo maior número de espécies e de populações de cada espécie. A elevação de grupos monofiléticos menores (geralmente conhecidos como seções de *Batrachospermum*) para o nível gênero tem sido uma clara tendência na taxonomia do grupo nos últimos anos. A antiga seção *Helminthoidea* foi elevada para gênero (*Sheathia*) baseado em caracteres morfológicos e análises filogenéticas de três marcadores moleculares (SALOMAKI *et al*., 2014). A seção *Setacea* foi também elevada a gênero por Rossignolo & Necchi (2016) com base em dados morfológicos e moleculares e o nome posteriormente alterado para *Torularia* (WYNNE, 2019). As seções relacionadas a seguir foram elevadas a gêneros em estudos recentes com base em evidências morfológicas e moleculares: *Virescentia* (NECCHI *et al*., 2018), *Acarposporophytum* (gênero *Acarposporophycos*, NECCHI *et al*., 2019a*)*, *Aristata* (gênero *Visia,* NECCHI *et al*., 2019a), *Macrospora* (gênero *Montagnia*, NECCHI *et al*., 2019b) e *Turfosa* (gênero *Paludicola*, VIS *et al.*, 2020). A tabela 1 resume os gêneros de Batrachospermales atualmente reconhecidos, as antigas seções a que pertenciam e a distribuição geográfica mundial.

Tabela 1. Gêneros da ordem Batrachospermales, família Batrachospermaceae.

Gêneros	Seções antigas	Distribuição	Referências
Acarposporophycos	*Acarposporophytum*	América do Sul	Necchi (1987), Necchi *et al*. (2019a)
Balliopsis	-	América do Sul, Africa e Ásia	Saunders & Necchi (2002)
Batrachospermum	-	Africa, América do Norte, América do Sul, Ásia, Australasia e Europa	Roth (1797), Kumano (2002), Guiry &Guiry (2020)
Kumanoa	*Contorta e Hybrida*	África, América do Norte, América do Sul, Ásia, Australasia e Europa	Sirodot (1873), Skuja (1931), Entwisle *et al*. (2009), Necchi & Vis (2012)
Lemanea	-	América do Norte, Ásia, Europa	Bory (1808)
Lympha		América do Norte	Evans *et al*. (2017)
Montagnia	*Macrospora*	África, América do Norte, América do Sul e Ásia	Kumano (1993), Necchi *et al*. (2019b)
Nocturama	*Batrachospermum*	América do Sul, Australásia	Entwisle *et al*. (2016)
Nothocladus	*Batrachospermum*	Africa, Ásia e Australásia	Skuja (1934), Schneider & Wynne (2007), Entwisle *et al*. (2016)
Paludicola	*Turfosa*	América do Norte, América do Sul, Ásia e Europa	Sirodot (1873), Vis *et al*. (2020)
Paralemanea	-	América do Norte, América do Sul, Ásia e Europa	Vis & Sheath (1992)
Petrohua	-	América do Sul	Vis *et al*. (2007), Schneider & Wynne (2007)
Psilosiphon	-	Australásia	Entwisle (1989)
Sheathia	*Helminthoidea*	África, América do Norte, Ásia, Australasia e Europa	Sirodot (1973), Salomaki *et al*. (2014)
Sirodotia	-	África, América do Norte, América do Sul, Australásia, Ásia e Europa	Kylin (1912), Lam *et al*. (2012)
Torularia	*Setacea*	Africa, América do Norte, América do Sul, Ásia, Australásia e Europa	Rossignolo & Necchi (2016), Wynne (2019)
Tuomeya	-	América do Norte	Harvey (1858), Kaczmarczyk *et al*. (1992), Kumano (2002)
Virescentia	*Virescentia*	América do Norte, América do Sul, Asia e Europa	Sirodot (1873), Necchi *et al*. (2018)
Visia	*Aristata*	América do Sul, Ásia e Australásia	Skuja (1933), Necchi *et al*. (2019a)
Volatus	-	América do Norte e Europa	Chapuis *et al*. (2017)

Fonte: Produção do autor

1.1. O gênero *Sirodotia*

Dentre as rodófitas continentais da ordem Batrachospermales, *Sirodotia* é um gênero amplamente distribuído no mundo todo (LAM *et al*., 2012). O gênero foi proposto por Kylin (1912) dentro de Batrachospermaceae com base na espécie tipo *S. suecica*. Apresenta morfologia similar ao gênero *Batrachospermum* e aos gêneros mais recentemente segregados como *Kumanoa* e *Virescentia* (ENTWISLE *et al*. 2009, LAM *et al*. 2012). O gênero *Sirodotia* se diferencia dos demais gêneros da família por apresentar carpogônio com base assimétrica (com uma protuberância) e carposporófito difuso que se estende ao longo do eixo principal, consistindo de filamentos gonimoblásticos indeterminados e prostrados, que produzem pequenos filamentos eretos determinados, sobre os quais se desenvolvem os carposporângios (NECCHI *et al*., 1993; KUMANO, 2002; NECCHI *et al*., 2007; LAM *et al*., 2012). As espécies do gênero têm sido reconhecidas com base em alguns caracteres morfológicos, como morfologia vegetativa do gametófito, presença de espermatângios em ramos especializados, posição do ramo carpogonial, origem dos filamentos gonimoblásticos em relação ao carpogônio e tamanho do carpogônio e carposporângio (NECCHI *et al*., 1993; KUMANO, 2002; NECCHI *et al*., 2007). Atualmente existem 13 espécies taxonomicamente aceitas em *Sirodotia* (KUMANO, 2002; OTT, 2009; GUIRY & GUIRY, 2020): *S. assamica*, *S. cirrhosa*, *S. delicatula*, *S. gardneri*, *S. huangshanensis*, *S. huillensis*, *S. iyengarii*, *S. kennedyi*, *S. polygama*, *S. segawae*, *S. sinica*, *S. suecica* e *S. yutakae.* Porém, poucas espécies foram analisadas com base em dados moleculares e morfológicos.

O gênero *Sirodotia* tem sido reconhecido como um grupo distinto e monofilético em vários estudos, tanto com base em dados morfológicos (NECCHI *et al*., 1993; KUMANO, 2002; NECCHI *et al*., 2007), quanto moleculares (VIS & SHEATH, 1999; LAM *et al*., 2012; PAIANO & NECCHI, 2013; JOHNSTON *et al*., 2014). Estudos moleculares sobre o gênero *Sirodotia* têm contribuído para melhor compreensão sobre a taxonomia infra-genérica (VIS & SHEATH, 1999; NECCHI *et al*., 2007; PAIANO & NECCHI, 2013; JOHNSTON *et al.*, 2014). Esses estudos mostraram que a diversidade genética é grande entre as espécies, levando à sinonimização de algumas espécies (*S. tenuissima* sinônimo de *S. suecica*) e à distinção genética e morfológica de outras (*S. huillensis* e *S. delicatula*). Além disso, esses estudos têm apontado a ocorrência de espécies crípticas com alto nível de divergência genética, porém sem a correspondente distinção morfológica, em alguns casos, apenas

geográfica. Em um estudo recente sobre o gênero *Sirodotia* (LAM *et al*., 2012) com a inclusão de um número expressivo de sequências de algumas regiões do globo foi encontrada provável espécie nova de amostra da África do Sul (referida como *S.* aff. *huillensis*). Paiano *et al*. (2013) por meio de estudos filogeográficos com *S. delicatula*, indicaram a existência de espécies crípticas no Brasil. Ademais, estudos moleculares realizados por Johnston *et al*. (2014) revelaram que os espécimes de *S. delicatula* da Malásia e do Brasil não são semelhantes geneticamente, sugerindo que o espécime da Malásia representa *S. delicatula*, enquanto que a espécie brasileira deve tratar-se de espécie distinta.

Os dados filogenéticos moleculares já apresentados para o gênero *Sirodotia,* assim como para outros gêneros e seções da ordem Batrachospermales, têm revelado numerosos casos de espécies crípticas (LAM *et al*., 2012; PAIANO & NECCHI, 2013; AGOSTINHO *et. al*., 2014; ROSSIGNOLO & NECCHI, 2016; NECCHI *et al*., 2019a, 2019b; VIS *et al*., 2019). Assim como nos gêneros e seções de *Batrachospermum*, as relações filogenéticas, limites de variação e diversidade genética entre espécies precisam de um estudo mais detalhado baseado em dados morfológicos e moleculares. A utilização de ferramentas moleculares para auxílio na taxonomia e biogeografia de algas tem contribuído no aprimoramento do esquema de identificação de táxons não somente nas descontinuidades na variação morfológica para distinguir espécies, mas com a utilização de dados genéticos e/ou geográficos (SAUNDERS, 2005). Dentre os gêneros de Batrachospermales que têm numerosas espécies, *Sirodotia* é um dos poucos que ainda não foi completamente revisado. Considerando este conjunto de informações e que poucas espécies têm sido reconhecidas mundialmente com base em dados moleculares e morfológicos o presente estudo foi proposto.

1.2. HIPÓTESES E OBJETIVOS GERAIS DO ESTUDO

1.2.1. Hipóteses

Com base nas informações atualmente disponíveis (morfológicas e moleculares) sobre representantes do gênero *Sirodotia* em âmbito mundial, as seguintes hipóteses gerais foram testadas:

1. O gênero *Sirodotia* será confirmado como grupo monofilético dentro da ordem Batrachospermales. Se a hipótese for corroborada, o gênero será reconhecido como

distinto dentro da ordem e confirmará conclusões de outros estudos filogenéticos (VIS *et al*., 1998; VIS & SHEATH, 1999; NECCHI *et al*., 2007; ENTWISLE *et al*., 2009; PAIANO & NECCHI 2013);

2. Espécies atualmente reconhecidas com base em caracteres morfológicos serão corroboradas pelos dados moleculares. E espécies novas, particularmente espécies crípticas, deverão ser encontradas e reconhecidas como espécies distintas dentro do gênero;

3. Os caracteres morfológicos atualmente usados para distinguir as espécies do gênero - presença de espermatângios em ramos especializados, posição do carpogônio que produz os filamentos gonimoblásticos e dimensões do carpogônio e carposporângio (NECCHI *et al*., 1993; KUMANO, 2002; NECCHI *et al*., 2007) – deverão ser corroborados pelos dados moleculares;

4. A biogeografia das espécies deverá ser um caráter adicional importante para a determinação de novas espécies, visto que os dados disponíveis indicam que algumas espécies apresentam nítida repartição geográfica.

1.2.2. Objetivos

Para testar estas hipóteses desenvolvemos o presente trabalho com os seguintes objetivos:

1. Inferir as relações filogenéticas das espécies dentro do gênero *Sirodotia* e com as de outras de outros gêneros da ordem Batrachospermales, com base na análise das sequências do gene plastidial que codifica a subunidade grande da RuBisCO (*rbc*L), da região de “barcode” do gene mitocondrial que codifica a subunidade 1 da citocromo *c* oxidase (COI-5P) e da região de “barcode” do gene nuclear que codifica a subunidade grande do ribossomo (LSU);

2. Fornecer subsídios para melhor definição dos limites de variação interespecífica com base na análise das sequências desses três marcadores;

3. Avaliar quais caracteres morfológicos têm valor diagnóstico, inclusive à luz dos novos dados moleculares, para a delimitação de espécies do gênero *Sirodotia* e circunscrever as espécies reconhecidas no gênero em âmbito mundial.

1.3. REFERÊNCIAS

AGOSTINHO, D. C; NECCHI, O. Jr. Systematics of the section *Virescentia* of the genus *Batrachospermum* (Batrachospermales, Rhodophyta) in Brazil. **Phycologia,** v.53, n. 5, 2014.

BHATTACHARYA, D.; YOON, H. S.; HACKETT, J. D. Photosynthetic eukaryotes unite: endosymbiosis connects the dots. **Bio Essays,** v. 26, p. 50-60, 2004.

BORY DE SAINT-VINCENT, [J.B.G.M.]. Mémoire sur le genre *Lemanea* de la famille des Conferves. **Annales du Muséum d'Histoire Naturelle**, v. 12, p. 177-190, 1808.

DE CLERCK, O.; BOGAERT, K.A.; LELIAERT, F. Diversity and evolution of algae: primary endosymbiosis. **Advances in Botanical Research,** v. 64, p. 55-8, 2012.

ENTWISLE, T.J. *Psilosiphon scoparium* gen. et sp. nov. (Lemaneaceae), a new red alga from south-eastern Australian streams. **Phycologia**, v. 28, p. 469-475, 1989.

ELORANTA P., KWANDRANS J., KUSEL-FETZMANN E. *Rhodophyta and Phaeophyceae*. In Freshwater Flora of Central Europe, Heidelberg: Spectrum Akademischer Verlag, p. 57–59, 2011.

ENTWISLE, T.J.; JOHNSTON, E.T.; LAM, D.W.; STEWART, S.A. & VIS, M.L. *Nocturama* gen. nov., *Nothocladus* s. lat. and other taxonomic novelties resulting from the further resolution of paraphyly in Australasian members of *Batrachospermum* (Batrachospermales, Rhodophyta). **Journal of Phycology,** v. 52, p. 384-396, 2016.

ENTWISLE, T. J.; VIS, M. L.; CHIASSON, W. B.; NECCHI, O. J.; SHERWOOD, A. R. Systematics of the Batrachospermales (Rhodophyta) - a synthesis. **Journal of Phycology,** v. 45, p. 704-715, 2009.

EVANS, J.R., CHAPUIS, I.S. & VIS, M.L. Adding to the freshwater red algal diversity in North America: *Lympha mucosa* gen. et sp. nov. (Batrachospermales, Rhodophyta). **Algae**, v. 32, p. 171-179, 2017.

FRANCESCHINI, I. M.; BURLIGA, A. L.; REVIERS, B. D.; PRADO, J. F.; RÉZIG, S. H. **Algas: Uma abordagem filogenética, taxonômica e ecológica**. Artmed Editora, 2010. Disponível em: https://books.google.com.br/books?id=XgEluMvQDZIC.

FRESHWATER, D.W.; FREDERICQ, S.; BUTLER, B.S.; HOMMERSAND, M.H.; CHASE, M.W. A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbc*L. **Proc. Natl. Acad. Sci. U. S. A.**, v. 91, p. 7281-7285, 1994.

GABRIELSON, P. W.; GARBARY, D. J.; SCAGEL, R. F. The nature of the ancestral red alga: inferences from a cladistic analysis. **Biosystems,** v. 18, p. 335–46, 1985.

GABRIELSON, P. W.; GARBARY, D. J.; SOMMERFELD, M. R.; TOWNSEND, R. A.; TYLER, P. L. **Phylum Rhodophyta. *In*: *Handbook of Protoctista: The Structure, Cultivation, Habitats and Life Histories of the Eukayotic Microorganisms and Their Descendants Exclusive of Animals, Plants and Fungi***. Jones and Bartlett Pub., Boston, 1990.

GARBARY, D. J.; GABRIELSON, P. W. **Taxonomy and evolution. In *Biology of the Red Algae***. Cambridge University Press, New York, 1990.

GRAHAM, L. E.; WILCOX, L. W. **Algae**. Prentice-Hall, New Jersey, 2009.

GUIRY, M.D. How many species of algae are there? **Journal of Phycology**, v.48, p. 1057-63, 2012.

GUIRY, M. D.; GUIRY, G. M. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org (accessed 18 July 2020).

GURGEL, C. F. D.; LOPEZ-BAUTISTA, J. **Red algae, *In*: Encyclopedia of Life Sciences**. Wiley, Chichester, UK, 2007.

HARVEY, W.H. *Nereis boreali-americana*: or, contributions to a history of the marine Algae of North America. Part III. Chlorospermeae. **Smithsonian Contributions to Knowledge,** v. 10, p. 1-140, 1858.

JOHNSTON, E. T.; LIM, P.E., BUHART, N.; KEIL, E. J.; DJAWAD, M. I.; VIS, M. L. Diversity of freshwater red algae (Rhodophyta) in Malaysia and Indonesia from morphological and molecular data. **Phycologia**, v. 53, p. 329-341, 2014.

KACZMARCZYK, D., SHEATH, R.G. & COLE, K.M. Distribution and systematics of the fresh water genus *Tuomeya* (Rhodophyta, Batrachospermaceae). **Journal of Phycology**, v. 28: 850-855, 1992.

KUMANO, S. Taxonomy of the family Batrachospermaceae (Batrachospermales, Rhodophyta). **Japanese Journal of Phycology,** v. 41, 253-272, 1993.

KUMANO, S. Freshwater red algae of the world. **Bristol, Biopress Ltd**, p. 375, 2002.

KYLIN, H. Studien über die Schwedischen Arten der Gattungen *Batrachospermum* und *Sirodotia* nov. gen. **Nova Acata Reg. Soc. Sci. Upsal**. Sér 4, v. 3, p. 1-40, 1912.

LAM, D., ENTWISLE, T.J., ELORANTA, P., KWANDRAS, J.; VIS, M.L. Circumscription of species in the genus Sirodotia (Batrachospermales, Rhodophyta) based on molecular and morphological data. **Europena Journal of Phycology,** v. 47, p. 42-50, 2012.

LAM, D. W., VERBRUGGEN, H., SAUNDERS, G. W. & VIS, M. L. Multigene phylogeny of the red algal subclass Nemaliophycidae. **Molecular Phylogenetic Evolution,** v. 94, p. 730–736, 2015.

LEE, R. E. **Rhodophyta, In: Phycology**. 4. ed. New York: Cambridge University Press, 2008. p. 89-132.

LE GALL, L.; SAUNDERS, G. W. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: establishing the new red algal subclass Corallinophycidae. **Molecular Phylogenetics and Evolution,** v. 43, p. 1118-30, 2007.

LOPEZ-BAUTISTA, J. M. **Red algal genomics: A synopsis. Cellular Origin, Life in Extreme Habitats and Astrobiology**. Springer; 1st Edition, 2010. 526.

MULLER, K. M.; OLIVEIRA, M. C.; SHEATH, R. G.; BHATTACHARYA, D. Ribosomal DNA phylogeny of the Bangiophycidae (Rhodophyta) and the origin of secondary plastids. **American Journal of Botany,** v. 88, p. 1390-400, 2001.

MUNÕZ-GOMEZ, S.A.; MEJÍA-FRANCO, F. G.; DURNIN, K.; COLP, M.; GRISDALE, C.J.; ARCHIBALD,J.M.; SLAMOVITS, C.H. The New Red Algal Subphylum Proteorhodophytina Comprises the Largest and Most Divergent Plastid Genomes Known. **Current Biology Report**, v. 27, p. 1677-1684, 2017.

NECCHI, O. JR. Studies on the freshwater Rhodophyta of Brazill – 3, *Batrachospermum brasiliense* sp. nov. from the state of São Paulo, southern Brazil. **Revista Brasileira de Biologia**, v. 47, p. 441-446, 1987.

NECCHI, O. JR. **Rhodophyceae.** In: BICUDO, C.E.M., MENEZES, M. (Eds.), Gêneros de algas de águas continentais do Brasil – chave para identificação e descrições. Ed. Rima, São Carlos, p. 505-516, 2017.

NECCHI, O. JR.; BRANCO, C.C.Z.; BRANCO, L.H.Z. Distribution of Rhodophyta in streams from São Paulo State, southeastern Brazil. **Archiv für Hidrobiology,** v. 147, p. 73-89, 1999.

NECCHI, O. JR., SHEATH, R. G., COLE, K. M. Distribution and systematics of the genus *Sirodotia* (Batrachospermales, Rhodophyta) in North America. **Journal of Phycology,** v. 29, p. 236-243, 1993.

NECCHI, O. JR.; VIS, M.L. **Monograph of the genus *Kumanoa* (Rhodophyta, Batrachospermales).** Stuttgart, J. Cramer in der GebrüderBorntraeger Verlagsbuchhandlung, 2012. 79 p. ISBN 9783443600433 (pbk.)0067-8112.

NECCHI, O. JR., VIS, M.L. & OLIVEIRA, M.C. Phylogenetic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North and South America. **Cryptogamie, Algologie,** v. 27, p. 117-127, 2007.

NECCHI, O. JR.; AGOSTINHO, D.C.; VIS, M. L. Revision of *Batrachospermum* section *Virescentia* (Batrachospermales, Rhodophyta) with the establishment of the new genus, *Virescentia* stat. nov. **Cryptogamie, Algologie**, v. 39, p. 313–338, 2018.

NECCHI, O. JR.; GARCIA, F.O. A. S.; PAIANO, M. O. Revision of *Batrachospermum* sections *Acarposporophytum* and *Aristata* (Batrachospermales, Rhodophyta) with the establishment of the new genera *Acarposporophycos* and *Visia.* **Phytotaxa,** v. 395, p. 051-065, 2019a.

NECCHI, O. JR.; GARCIA FO., A. S.; PAIANO, M. O.; VIS, M. L. Revision of *Batrachospermum* section *Macrospora* (Batrachospermales, Rhodophyta) with the establishment of the new genus *Montagnia*. **Phycologia,** v. 58, p. 582–91, 2019b.

NORTON, T. A.; MELKONIAN, M.; ANDERSEN, R. A. Algal biodiversity. **Phycologia,** v. 35, p. 308-326, 1996.

PAIANO, M. O.; NECCHI, O. JR. Phylogeography of the freshwater red alga *Sirodotia* (Batrachospermales, Rhodophyta) in Brazil. **Phycological Research**, v. 61, p. 249-255, 2013.

PAIANO, M. O.; CORTONA, A. D.; COSTA, J. F.; LIU, S. L.; VERBRUGGEN, H.; CLERCK, O. D. NECCHI, O. Jr. Organization of plastid genomes in the freshwater red algal order Batrachospermales (Rhodophyta). **Journal of Phycology**, v. 54, p. 25-33, 2018.

PASCOALOTO, D.; NECCHI, O. JR. Seasonal variation of *Sirodotia delicatula* Skuja (Rhodophyta, Batrachospemaceae) in a small stream in São Paulo State, southeastern Brazil. **Revista Brassileira de Biologia**, v. 50, p. 37-44, 1990.

PUESCHEL, C. M.; COLE, K. M. Rhodophycean pit plugs: an ultrastructural survey with taxonomic implications. **American Journal of Botany,** v. 69, p. 703–20, 1982.

QIU, H.; PRICE, D.C.; YANG, E.C.; YOON, H.S.; BHATTACHARYA, D. Evidence of ancient genome reduction in red algae (Rhodophyta). **Journal of Phycology**, v. 51, p. 624-36, 2015.

RODRIGUEZ-EZPELETA, N.; BRINKMANN, H.; BUREY, S. C.; ROURE, B.; BURGER, G.; LOFFELHARDT, W.; BOHNERT, H. J.; PHILIPPE, H.; LANG, B. F. Monophyly of primary photosynthetic eukaryotes: green plants, red algae, and glaucophytes. **Current Biology,** v. 15, n. 14, p. 1325-30, 2005.

ROSSIGNOLO, N. L. & NECCHI, O. Jr. Revision of section *Setacea* of the genus Batrachospermum (Batrachospermales, Rhodophyta) with emphasis on specimens from Brazil. **Journal of Phycology,** v. 55 (4), p. 337-346, 2016.

ROTH, A.W. *Bemerkungen über das Studium der cryptogamischen Wassergewächse*. pp. [1]-109, [+1 Druckfehler]. Hannover: bei den Gebrüdern Hahn. 1797.

SALOMAKI, E., KWANDRANS, J., ELORANTA, P., VIS, M. Molecular and morphological evidence for *Sheathia* gen. nov. (Batrachospermales, Rhodophyta) and three new species. **Journal of Phycology**, v. 50 (3), p. 526-542, 2014.

SAUNDERS, G. W. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future application. **Philosophical Transactions of the Royal Society,** B360, p. 1879–1888, 2005.

SAUNDERS, G. W.; HOMMERSAND, M. H. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. **American Journal of Botany,** v. 91, n. 10, p. 1494-507, 2004.

SAUNDERS, G.W. & NECCHI, O., Jr. Nuclear rDNA sequences from *Ballia prieurii* support recognition of *Balliopsis* gen. nov. in the Batrachospermales (Florideophyceae, Rhodophyta). **Phycologia**, v. 41, p. 61-67, 2002.

SCHNEIDER, C.W. & WYNNE, M.J. A synoptic review of the classification of red algal genera a half a century after Kylin's "*Die Gattungen der Rhodophyceen*". **Botanica Marina**, v. 50, p. 197-249, 2007.

SCOTT, J.; YANG, E.C.; WEST, J.A.; YOKOYAMA, A.; KIM, H.J.; SUSAN, L. DE GOËR; O'KELLY, C.J.; ORLOVA, E.; KIM, S.Y.; PARK, J.K.; YOON, H.S. On the genus Rhodella, the emended orders Dixoniellales and Rhodellales with a new order Glaucosphaerales (Rhodellophyceae, Rhodophyta). **Algae**, v. 26, p. 277-288. 2011.

SHEATH, R. G. **The biology of freshwater red algae. In *Progress in Phycological Research***. Biopress, Bristol, 1984.

SIRODOT, S. Nouvelle classification des algues d'eau douce du genre *Batrachospermum*: développement; générations alternates. **Comptes-rendus**

hebdomadaires des séances de l'Académie des Sciences, v. 76, p. 1216-1120, 1873.

SKUJA, H. Untershuchungen über die Rhodophyceen des Süsswassers. **Archiv für Protistenkunde**, v. 80, p. 357-366, 1933.

SKUJA, H. Untersuchungen über die Rhodophyceen des Sußwassers. **Beihefte zum Botanische Centralblatt,** v. 52, p. 173-192, 1934.

VIS, M.L. & SHEATH, R.G. Systematics of the freshwater red algal family Lemanaceae in North America. **Phycologia**, v. 31, p. 164-179, 1992.

VIS, M.L. & SHEATH, R.G. A molecular investigation of the systematic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North America. **Phycologia**. v. 38, p. 261-266, 1999.

VIS, M.L.; HARPER, J.T. & SAUNDERS, G.W. Large subunit rDNA and rbcL gene sequence data place *Petrohua bernabei* gen. et sp. nov. in the Batrachospermales (Rhodophyta), but do not provide further resolution among taxa in this order. **Phycological Research**, v. 55, p. 103-112, 2007.

VIS, M.L.; LEE, J.; ELORANTA, P.; CHAPUIS, I.S.; LAM, D.W. & NECCHI, O. JR. *Paludicola* gen. nov. and revision of the species formerly in *Batrachospermum* section *Turfosa* (Batrachospermales, Rhodophyta). **Jornal of Phycology**, p. 1-18, 2020.

WYNNE, M.J. *Torularia* Bonnnemaison, 1828, a generic name to be reinstated for *Atrophycus* Necchi & Rossignolo, 2017. **Notulae Algarum**, v. 89, p. 1-4, no figs, 2019.

YANG, E.C; SCOTT, J.; WEST, J.A.; ORLOVA, E.; GAUTHIER, D.; KÜPPER, F.C.; YOON H.S.; KARSTEN, U. New taxa of the Porphyridiophyceae (Rhodophyta): Timspurckia oligopyrenoides gen. et sp. nov. and Erythrolobus madagascarensis sp. nov**. Phycologia**, v. 49, p. 604-616, 2010.

YOON, H. S.; HACKETT, J. D.; CINIGLIA, C.; PINTO, G.; BHATTACHARYA, D. A molecular timeline for the origin of photosynthetic eukaryotes. **Molecular Biology and Evolution,** v. 21, p. 809-818, 2004.

YOON, H. S., MÜLLER, K. M., SHEATH, R. G., OTT, F. D., BHATTACHARYA, D. Defining the Major Lineages of Red Algae (Rhodophyta). **Journal of Phycology,** v. 42, p. 482-492, 2006.

2. CAPITULO 1

Molecular and morphological evidence for a new species in the genus *Sirodotia* (Batrachospermales, Rhodophyta) from the State of Assam, India

Natalia L. ROSSIGNOLO, Farishta YASMIN, John A. WEST,

E.K. GANESAN & Orlando NECCHI Jr

Phytotaxa 437 (3): 121–134

https://doi.org/10.11646/phytotaxa.437.3.1

https://www.mapress.com/j/pt/

ISSN 1179-3155 (print edition)

ISSN 1179-3163 (online edition)

2.1. Abstract

In India the genus *Sirodotia* has been documented solely on morphological data. A new species of *Sirodotia* (*Sirodotia assamica sp. nov.*) was found in two localities in the State of Assam, India. Phylogenetic relationships of the new species were inferred on the basis of DNA sequence data for the plastid *rbc*L gene and the barcode region of the mitochondrial COI- 5P gene. Taxonomic affinities of the new species were determined by morphological analyses and a distinctive character was found for this species: spermatangia arranged in clusters. Although this character is also observed in *S. huillensis*, both species are genetically highly divergent (4.5–5.0% for *rbc*L and 9.6–10.1% for COI-5P). DNA sequences from Indian specimens formed a well-supported clade, sister to *S. delicatula* from Malaysia. DNA sequence divergence between S. *assamica* and *S. delicatula* varied from 2.5–2.7% for *rbc*L and COI-5P. Intraspecific divergence between the two sequences from India were low (0.4–0.5%). A full description and photographs of the new species are provided, as well as a comparison with morphologically similar and phylogenetically allied species reported from India and other Asian regions.

Keywords: Batrachospermales**,** COI-5P**,** freshwater Rhodophyta, India**,** molecular systematics, *rbc*L

2.2. Introduction

The genus *Sirodotia* is a member of the freshwater red algal order Batrachospermales (Entwisle *et al*. 2009) and was proposed by Kylin (1912) based on the type species *S. suecica*. *Sirodotia* currently includes species with similar vegetative morphology to the genus *Batrachospermum* and other recently segregate genera such as *Kumanoa*, *Virescentia* and *Torularia* (Entwisle *et al.* 2009; Lam *et. al*., 2012). *Sirodotia* is distinguished by two important reproductive characters: an asymmetrical carpogonium base (with a protuberance), and a diffuse carposporophyte extending along the main axis consisting of indeterminate, prostrate gonimoblast filaments with short erect branches producing carposporangia (Necchi *et al*., 1993, 2007; Kumano, 2002; Lam *et al*., 2012). Within *Sirodotia*, species are differentiated based on the morphology of the gametophyte, presence of spermatangia on specialized branches, the origin of the carpogonial branch, which side of the carpogonium (protuberant or

non-protuberant) produces the gonimoblast filaments, and the size of the carpogonium and carposporangia (Necchi *et al*., 1993; Kumano, 2002; Necchi *et al.*, 2007). Kumano (2002) recognized eight species worldwide, but there are eleven species currently taxonomically accepted (Guiry & Guiry, 2020). Only three geographically widespread species have been reported: *S. suecica* Kylin, *S. huillensis* Welwitsch ex West & G.S.West and *S. delicatula* Skuja (Kumano, 2002; Necchi *et al*. 2007; Lam *et al*., 2012). *Sirodotia* species occur on all continents except Antarctica and have been reported from various locations in North America, South America, Australasia, and Europe, with sparse reports in China, India, Southeast Asia, and Africa (Israelson, 1942; Balakrishnan & Chaugule, 1980; Kumano, 1982; Necchi *et al*., 1993; Entwisle & Foard, 1999; Sheath & Sherwood, 2002; Carmona *et al*., 2006; Eloranta & Kwandrans, 2007; Necchi *et al*., 2007; Eloranta *et al.*, 2011; Lam *et al*., 2012).

The use of DNA sequence data for genes such as the plastid RuBisSCO large sub-unit (*rbc*L) and the mitochondrial cytochrome c oxidase sub-unit (COI-5P) in conjunction with morphological data, has provided better species circumscriptions, insights into taxonomically informative characters and phylogeographic relationships for members of the Batrachospermales and Rhodophyta in general (Vis & Sheath, 1999; Necchi *et al.*, 2007; Lam *et al*., 2012). Despite the many geographically widespread records, only a few *Sirodotia* species have been recognized worldwide based on both molecular and morphological data: *S. suecica* Kylin (temperate regions of North America and Europe), *S. delicatula* Skuja (South America, Japan, and Southeast Asia) and *S. huillensis* W. West & G.S. West (tropical and subtropical regions of the Americas and Asia) (Necchi, 1991; Necchi *et al*., 1993, 2007; Vis & Sheath, 1999; Lam *et al.,* 2012). Molecular studies of *Sirodotia* have provided insights into the infrageneric taxonomy. Vis and Sheath (1999) analyzed *S. suecica, S. tenuissima*, and *S. huillensis* from North America and proposed *S. tenuissima* to be synonymous with *S. suecica* but kept *S. huillensis* as a distinct species. Recently, DNA sequence data for *S. delicatula* from Brazil have shown this species to be morphologically and genetically distinct from *S. huillensis* and *S. suecica* (Necchi *et al*., 2007). Further molecular studies of *Sirodotia delicatula* from Brazil indicated the existence of cryptic species (Paiano & Necchi, 2013). Johnston *et al*. (2014) showed *S. delicatula* from Malaysia and Brazil to be genetically distinct. Considering the proximity to the type locality (Indonesia) of *S. delicatula*, they suggested that the

Malaysian specimen represents *S. delicatula*, while the Brazilian species most likely is a new species.

The genus *Sirodotia* has been collected in India and species identified using morphological observations. Biswas (1934, 1949) reported *Sirodotia cirrhosa* Skuja ex M.S.Balakrishnan & B.B.Chaugule from north-east India based on the identification by Skuja (Ganesan *et al*., 2018). *Sirodotia cirrhosa* was synonymized with *S. huillensis* by Kumano (2002). However, *Sirodotia cirrhosa* has been listed as a distinct species by Guiry & Guiry (2020). Three other species of *Sirodotia* have been collected and described in India: *S. huillensis* (Welwitsch ex West & G.S.West) Skuja, *S. iyengarii* Baluswami & Babu (1999) and *S. moniliformis* Skuja (Ganesan *et al*., 2018). Specimens from India identified as *S. huillensis* and *S. iyengarii* are probably not attributable to *Sirodotia* because some morphological characteristics (twisted or contorted carpogonial branches, gonimoblast developing from the hypogynous cell and compact arrangements of carposporophytes) in their descriptions are not consistent with the current genus circumscription. *Sirodotia moniliformis* Skuja is of uncertain taxonomic status (Ganesan *et al*., 2018).

Recent Indian Batrachospermales collections allowed us to assess their true diversity and to compare these collections with previously described species using newly generated morphological and DNA sequence data. This study describes a new species of *Sirodotia* found in the State of Assam, India, based on morphological and molecular evidence.

2.3. Material and methods

Three samples of *Sirodotia* were collected from two localities in the State of Assam, India, for morphological and molecular analyses (Table 2). Voucher specimens were deposited in the Herbarium SJRP (São Paulo State University, Campus of São José do Rio Preto, SP, Brazil). The diagnostic characters currently applied in the genus *Sirodotia* (see Necchi, 1991; Necchi *et al*., 1993; Vis & Sheath, 1999; Kumano, 2002; Necchi *et al*., 2007; Lam *et al.,* 2012) were used for morphological analyses. All measurements were made as described by Necchi (1990). For observations and measurements, we used an Olympus SZH10 stereoscope and an Olympus BH-2 light microscope (Olympus Corp., Tokyo, Japan). Photomicrographs were taken with a Leica DFC 320 digital camera using LAS (Leica

Application Suite) capture and image analysis software coupled to a Leica DM5000 microscope (Leica Microsystems, Wetzlar, Germany).

Protocols for DNA extraction, PCR amplification, purification and DNA sequencing were described in our previous studies: Rossignolo & Necchi (2016), Necchi *et al*. (2018; 2019). DNA sequences were generated for two molecular markers: the plastid-encoded RuBisCO large-subunit gene (*rbc*L), and the barcode region of the mitochondrial encoded cytochrome c oxidase subunit 1 (COI-5P). Analyses were conducted including DNA sequence data from previously published studies (Tables S1-S2) available in GenBank (Benson *et al*., 2013). For COI-5P sequence data (664 bp), distance analysis using Neighbor Joining (NJ) and TIM2+G (Transition model, unequal base frequency and Gamma parameter) as model determined as the best-fit model of sequence evolution by the corrected Akaike Information Criterion (cAIC) by jModeltest 2.1.4 (Darriba *et al*., 2012) performed with Molecular Evolutionary Genetics Analysis (MEGA version X, Kumar *et al*., 2018) with 1,000 bootstrap replicates.

For phylogenetic analyses of the *rbc*L sequence data (1,282 bp), a GTR + I + G was determined by cAIC using jModelTest 2.1.4. Maximum likelihood (ML) topologies and bootstrap values from 10,000 bootstrap replicates were inferred using RAxML graphic user interface version 1.5 (Silvestro & Michalak, 2012). Bayesian analysis (BA) was performed in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) with three runs of five chains of Metropolis coupled Markov Chain Monte Carlo (MCMC) for 10×10^6 generations saving one tree every 1000 generation. The first 500 trees were removed as burn-in prior to determining the posterior probabilities. Burn-in cutoff was estimated in Tracer 1.7.1 (Rambaut *et al.*, 2018).

TABLE 2. Description of collecting sites and GenBank accession numbers for samples of *Sirodotia assamica* sp. nov. from India analyzed in this study.

Species/code	Location, date and herbarium number	GenBank accession	
		COI-5P	*rbc*L
Sirodotia sp. – IN1a	India: Assam, Nagaon District, Chapanala; 26º19'13.7"N, 92º10'16.5" E; O. Necchi Jr., J.A. West, E.K. Ganesan & F. Yasmin, 23.ii.2018 (SJRP 32583).	MN508239	MN496129
Sirodotia sp. – IN1b	India: Assam, Nagaon District, Chapanala; 26º19'13.7"N, 92º10'16.5" E; F. Yasmin, 25.ii.2019 (SJRP 32584)	-----	-----
Sirodotia sp. – IN2	India: Assam, Sonapur, Tegheria; 26º05'52" N, 92º02'02"; F. Yasmin, 24.ii.2019 (SJRP 32585).	MN508240	MN496130

Source: Author's own production

2.4. Results and discussion

2.4.1. Molecular analyses

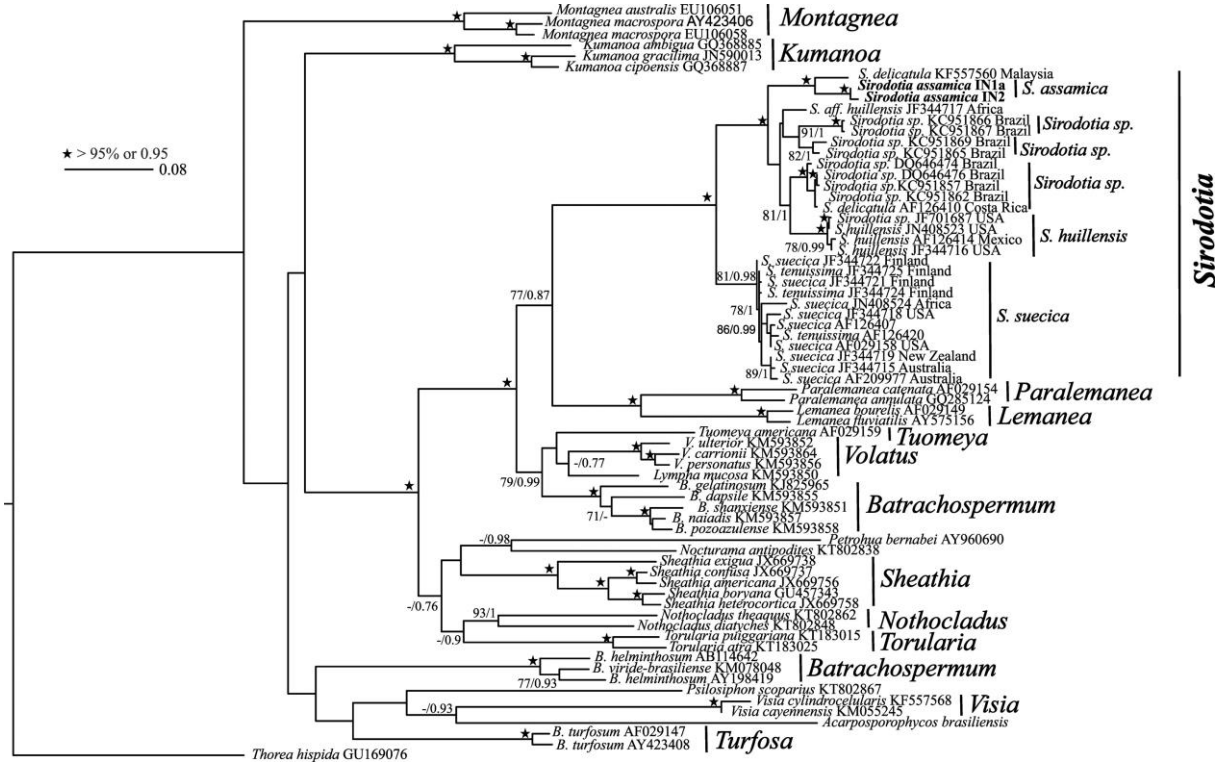
Two *rbc*L and two COI-5P sequences were newly generated in this study (Table 2). These sequences were compared to GenBank DNA sequences (68 of *rbc*L and 12 of COI-5P) from specimens collected in Africa, Australia, Brazil, Costa Rica, Finland, India, Malaysia, Mexico, New Zealand and USA (Table S1, S2). Both the BA and ML analyses of the *rbc*L sequences resulted in the same tree topology such that only the ML tree is shown (Fig. 2). Phylogenetic analyses showed the genus *Sirodotia* as a well-supported clade with two major subclades: the first comprising *S. suecica* specimens from several regions of the world (Africa, Australia, Finland, USA and New Zealand) and the second containing all remaining specimens, including the *Sirodotia assamica sp. nov.* specimens from India (Fig.2). This second major subclade contains sequences identified as *S. huillensis* (from Mexico and the USA), *Sirodotia spp*. from Brazil, *S.* aff. *huillensis* (from Africa), and *S. delicatula* (from Malaysia) (Fig. 2, Table S1). The DNA sequences from Brazil represent three new species yet to be described (Paiano & Necchi 2013). Our specimens from India formed a well-supported clade sister to *S. delicatula* from Malaysia. The divergence

between DNA sequences from India and *S. delicatula* was high (2.5–2.7%), whereas the intraspecific divergence between the two Indian sequences was low (0.4%). Divergence levels among the sequences from India and Malaysia indicate that they represent two separate species. The sequence from Malaysia is considered the true *S. delicatula* because the specimens were collected near Indonesia, the type locality (Johnston *et al.* 2014).

The COI-5P NJ tree (Fig. 3, Table S2) showed two well-supported groups: a group comprised of *S. suecica* DNA sequences from Africa, Canada, and USA; and a group containing DNA sequences of the remaining species: *Sirodotia* spp. (Brazil), *S. huillensis* (USA), and the two sequences from India. The DNA sequences from India formed a clade with high support representing a distinct species with high interspecific divergence (6.9–10.1%) to the other species and a low intra-specific divergence (0.5%).

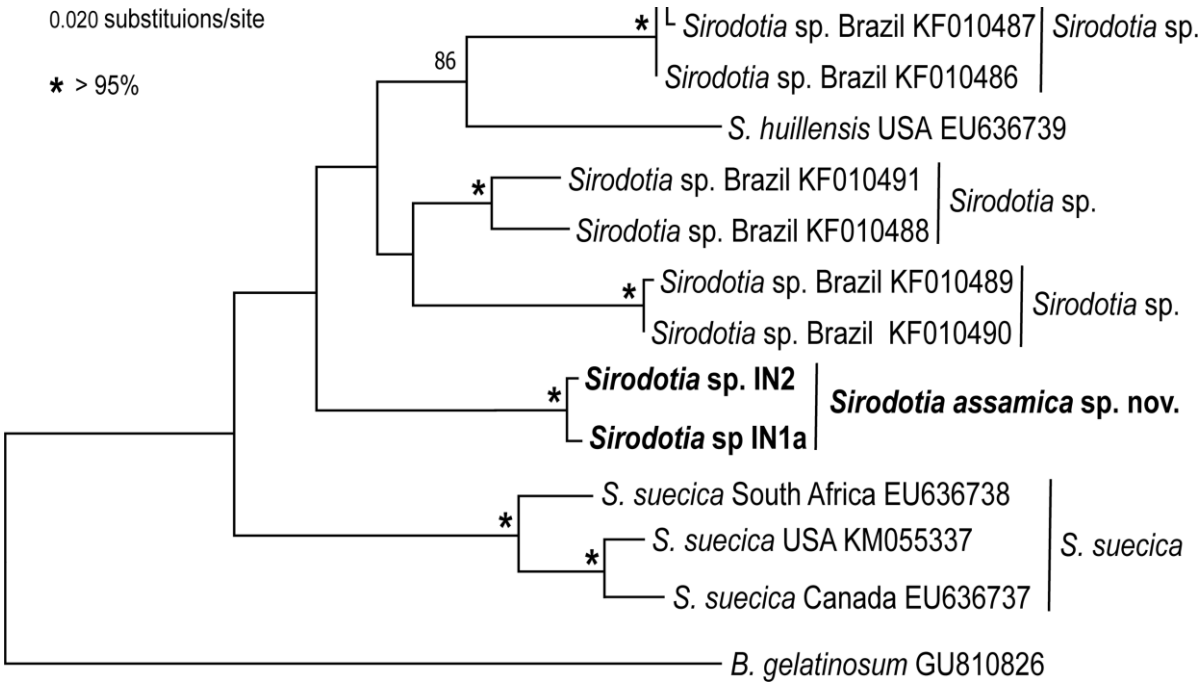
The sequence divergences observed in *rbc*L and COI-5P from species of *Sirodotia* (0.4% and 0.5%) are within the intraspecific variation previously observed in others species of the order Batrachospermales (Agostinho *et al*. 2014; Rossignolo & Necchi 2016; Necchi *et al*. 2018, 2019). These data indicate that the DNA sequences from India represent a distinct species that is described below.

FIGURE 2. Maximum likelihood phylogenetic tree based on rbcL DNA sequences. The numbers associated with the nodes indicate the bootstrap values (BS) for maximum likelihood and posterior probability (PP) for Bayesian analysis, respectively. Nodes without values indicate BS ≤ 70% and PP ≤ 0.70. Sample codes as in Table 2.



Source: Author's own production

FIGURE 3. Distance tree (neighbor joining) of COI-5P DNA sequences for Sirodotia species and Batrachospermales outgroup taxa. The numbers associated with the nodes indicate the bootstrap values, nodes without values indicate support < 70%. Sample codes as in Table 2.



Source: Author's own production

2.4.2. Morphological analyses and taxonomic proposals

Sirodotia assamica Necchi, Rossignolo, Yasmin, West and Ganesan ***sp. nov.*** (Fig. 4 a–h)

Description. Plants dioecious or monoecious, irregularly branched; apices straight; apical cells protruse. Whorls dense or loose, pear-shaped or obconical, contiguous, 400–665 µm in diameter. Internodes 547–1177 µm in length. Cortical filaments well-developed, with two to four layers. Primary fascicles straight, 6–12 cell storeys; proximal cells cylindrical or ellipsoidal, 17–42 µm in length, 7–13 µm in diameter; distal cells obovoidal, ellipsoidal or sub-spherical, 9–14 µm in length, 5–9 in diameter; di- or trichotomously branched. Secondary fascicles abundant, covering the entire internode, equal to or shorter than the length of the primary fascicles.

Spermatangia spherical, terminal or sub-terminal, arranged in clusters on primary or secondary fascicles, 6–8 µm wide. Carpogonial branches straight or slightly curved, short, 7–23 µm long, composed of 1–5(–6) disc- or barrel-shaped cells, 5–9 µm long, 4–8 µm wide, arising from periaxial cells of primary fascicles; involucral

filaments few and short, composed of 1–3 short-cylindrical or ellipsoidal cells. Carpogonia asymmetrical, with a hemispherical protuberance on one side of the basal portion, 37–64 µm in length, 10–16 µm in diameter; trichogynes elongate-cylindrical, ellipsoidal or lageniform, sessile or pedicellate.

Carposporophyte diffuse with no definite shape. Gonimoblast initial developing from protuberant side of the carpogonium; gonimoblast filaments prostrate, creeping among fascicles or cortical filaments along the main axis; with erect branches of 1–4 cylindrical or ellipsoidal cells, producing carposporangia at the tips; carposporangia obovoidal or sub-spherical, 11–14 µm in length, 6–8µm in diameter.

Holotype: India, Assam, Nagaon District, Chapanalla; 26º19’13.7” N, 92º10’16.5” E; coll. F. Yasmin, 25.ii.2019 (SJRP 32584). Holotype specimen not sequenced.

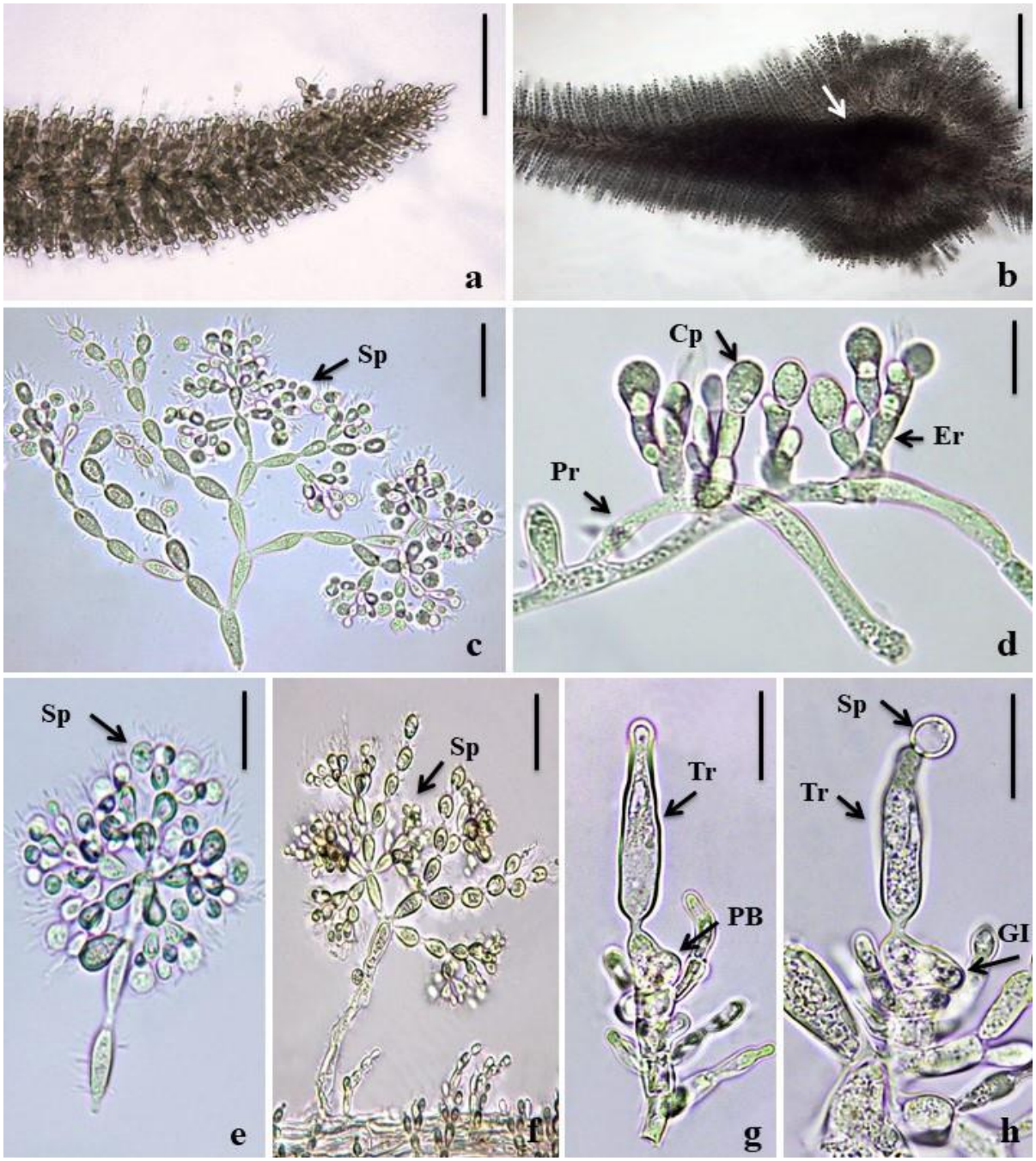
Paratypes: India, Assam, Sonapur, Tegheria; 26º05’52” N, 92º02’02” E; coll. F. Yasmin, 24.ii.2019 (SJRP 32583); India, Assam, Nagaon District, Chapanalla; coll. O. Necchi Jr. *et al*., 23.ii.2018 (SJRP 32585).

DNA sequences: COI-5P (MN508239, MN508240) and *rbc*L (MN496129, MN496130).

Remarks: *Sirodotia assamica* most distinguishing feature is the occurrence of spermatangia arranged in clusters, thus far described only for *S. huillensis*. However, these two species are genetically highly divergent (4.5%–5.0% for *rbc*L and 9.6%–10.1% for COI-5P). *Sirodotia assamica* is most closely comparable to *S. cirrhosa, S. delicatula* and *S. sinica* based on the vegetative and reproductive characteristics and the occurrence in or near India (Table 3). *Sirodotia assamica* differs from *S. sinica* in having longer carpogonia (37–64 µm versus 20–41 µm in length, respectively), by the arrangement of spermatangia in clusters and smaller carposporangia (11–14 µm x 6–8 µm versus 12–18 µm x 8–10 µm, respectively). *Sirodotia assamica* differs from *S. delicatula* in having larger whorls (400–665 µm versus 137–306 µm in diameter, respectively), greater number of cell storeys in primary fascicles (6–12 versus 4–6 cells, respectively), longer carpogonia (37–64 µm x 25–40 µm in length, respectively) and spermatangia in clusters. Moreover, they are genetically quite divergent (2.5–2.7% for *rbc*L and 4.2%–4.7% for COI-5P). *Sirodotia cirrhosa* was described by Balakrishnan & Chaugule (1980) from northern and southern India. However, *S. cirrhosa* differs from *S. assamica* in having shorter carpogonia (37–64 µm x 29–41 µm long) and smaller carposporangia (11–14 µm x 8–11 µm long) and lacking

spermatangia arranged in clusters. There are no molecular data for comparison since *S. cirrhosa* has not been reported since the record by Balakrishnan & Chaugule (1980). Moreover, the nomenclatural type of *S. cirrhosa* is not available for a comparative analysis since it is not lodged in the UPS herbarium (Uppsala University, Sweden), where it was supposed to be deposited according to Balakrishnan & Chaugule (1980).

FIGURE 4. *Sirodotia assamica sp. nov.* **a.** Main axis. **b.** Whorl showing densely arranged gonimoblast filaments along the internode (arrow). **c.** Primary fascicles and spermatangia (Sp) in clusters on a primary fascicle. **d.** Carposporangia (Cp), prostrate (Pr) and erect (Er) gonimoblast filaments. **e.** Detail of spermatangia (Sp) in clusters on primary fascicle. **f.** Spermatangia (Sp) in clusters on a secondary fascicle. **g.** A carpogonium with a mature trichogyne (Tr) and a basal hemispherical protuberance (PB). **h.** Fertilized carpogonium with attached spermatium and gonimoblast initial (GI) on the same side of the basal protuberance. Scale bar: 100 µm for figure a; 50 µm for figures b, c and f; 20 µm for figures d,e,g and h.



Source: Author's own production.

TABLE 3. Comparative morphology and distribution of *Sirodotia* species.

Morphological characters	*S. assamica*	*S. cirrhosa*	*S. delicatula*	*S. huillensis*	*S. sinica*
Whorls	Separated, 400–665 µm wide	95–540 µm wide	Separated, 137–306 µm wide	Separated, 162–540 µm wide	Separated
Primary fascicles	6–12 cells	5–9 cells	4–6 cells	3–12 cells	7–10 cells
Carpogonium length	37–64 µm long,10–16 µm wide	29–41µm long, 6,5-8,5 µm wide	25–40 µm long	28,5–53 µm long	20–41 µm long
Carpogonial branches	7–23 µm long, 1–5 (-6) disc- or barrel-shaped cells	3–5 barrel-shaped cells	8–15 µm long, 3–5 barrel-shaped cells	2–5 barrel-shaped cells	10–20 µm lenght, 2–6 barrel-shaped cells
Origin of the gonimoblast filaments	on the protuberant side of the carpogonium	on the protuberant side of the carpogonium	on the protuberant side of the carpogonium	on the protuberant side of the carpogonium	on the protuberant side and the opposite side of the carpogonium
Spermatangia	spherical, terminal or sub-terminal, in clusters, 6–8 µm wide	subglobular, terminal, singly or in pairs, 6–7 µm wide	Terminal, spherical, 4–8 µm wide	Terminal, sub-terminal, in clusters or alone, spherical, 5–8 µm wide	terminal on fascicles, 5–9 µm wide
Carposporangia	terminal, obovoidal, 11–14 µm long, 6–8 µm wide	ellipsoidal to obovoidal, 8–11 µm long, 5.5–8 µm wide	spherical, 6–8 µm wide	Obovoidal, 10–13 µm long, 6–8 µm wide	Ovoidal or obovoidal, 12–18 µm long 8–10 µm wide
Geographic distributio	India - Assam (Asia)	India - Assam, Burma India, Java and South India (Asia)	Indonesia, Malaysia (Asia)	Angola (Africa), India, Malaysia, Indonesia (Asia), USA, Mexico (North America)	China, Japan (Asia)
References	This study	Balakrishnan & Chaugule (1980)	Skuja (1938), Kumano (1982, 2002), Necchi *et. al.* (1993)	Skuja (1931), Balakrishnan & Chaugule (1980), Kumano (2002), Necchi *et al*. (2007), Elaya Perumal *et al*. (2015)	Jao (1941), Kumano (2002)

Source: Author's own production.

2.5. Conclusion

Sirodotia assamica is here proposed as a new species from the northeastern State of Assam in India based on molecular and morphological evidence. The most distinctive morphological character for the new species is the spermatangia arranged in clusters. Although this character is also observed in *S. huillensis*, both species are genetically highly divergent (4.5–5.0% for *rbc*L and 9.6–10.1% for COI-5P). The specimens from India formed a well-supported clade sister to *S. delicatula* from Malaysia. The divergence between DNA sequences from India and *S. delicatula* was high for *rbc*L (2.5–2.7%), whereas the intraspecific divergence between the two Indian sequences was low (0.4%). The DNA sequences for COI-5P from India formed a clade with high support representing a distinct species with high interspecific divergence (6.9–10.1%) to the other species and a low intraspecific divergence (0.5%).

Acknowledgements

Rossignolo and Necchi are grateful to the Brazilian agencies FAPESP (2016/07808-1, 2016/16320-2) and CNPq (302415/2017-3) for financial support by grants and scholarship and to Morgan L. Vis, Ohio University, for the review of a preliminary version of the manuscript. Yasmin thankfully acknowledge the financial support of Department of Biotechnology, Government of India through Institutional Biotech Hub (No. BT/04/NE/2009).

2.6. References

Agostinho, D.C. & Necchi, O. Jr. (2014) Systematics of the section *Virescentia* of the genus *Batrachospermum* (Batrachospermales, Rhodophyta) in Brazil. *Phycologia* 53: 561–570. https://doi.org/10.2216/PH14-034.1

Balakrishnan, M.S. & Chaugule, B.B. (1980) Indian Batrachospermaceae. *In:* Desikachary, T.V. & Raja Rao, T.N. (Eds.) *Taxonomy of Algae*, University of Madras, Chennai, pp. 223–248.

Baluswami, M. & Babu, M. (1999) The structure and reproduction of *Sirodotia iyengarii sp. nov. In:* Subba-Rangiah, G. (Ed.) *Recent Trends in Algal Research*. Department of Botany, Andhra University, Waltair, pp. 237–244.

Benson, D.A., Cavanaugh, M., Clark, K., Ostell, J. & Wheeler, D.L. (2013) GenBank. *Nucleic acids Research* 41: 36–42. https://doi.org/10.1093/nar/gks1195

Biswas, K. (1934) Observations on algal collections from Khasia and Jaintia Hills, Assam, India. *Hedwigia* 74: 1–28.

Biswas, K. (1949) Common fresh and brackish water algal flora of India and Burma.vol. 2. *Records of the Botanical Survey of India* 15: 1–106.

Carmona, J.J., Montejano, G.Z. & Necchi, O. Jr. (2006) The ecology and morphological characterization of gametophyte and "Chantransia" stage of *Sirodotia huillensis* (Batrachospermales, Rhodophyta) from a stream in Central Mexico. *Phycological Research* 54: 108– 115. https://doi.org/10.1111/j.1440-1835.2006.00417.x

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Elaya Perumal, U., Uma Rani, V. & Chaugule, B.B. (2015) Studies on a red alga *Sirodotia huillensis* (Batrachospermaceae) from Orissa State, India. *Journal of Theoretical and experimental Biology* 11: 45–50.

Eloranta, P. & Kwandrans, J. (2007) Freshwater red algae (Rhodophyta): identification guide and to European taxa, particularly to those in Finland**.** *Norrlinia* 15:1–103.

Eloranta, P., Kwandrans, J. & Kusel-Fetzmann, E. (2011) Rhodophyta and Phaeophyceae. In Freshwater Flora of Central Europe, *Heidelberg*: Spectrum Akademischer Verlag, 57–59.

Entwisle, T.J. & Foard, H.J. (1999) *Sirodotia* (Batrachospermales, Rhodophyta) in Australia and New Zealand. *Australian Systematic Botany* 10: 605–613. https://doi.org/10.1071/SB98022

Entwisle, T.J., Vis, M.L., Chiasson, W.B., Necchi, O. Jr. & Sherwood, A.R. (2009) Systematics of the Batrachospermales—A Synthesis. *Journal of Phycology* 45: 704–715. https://doi.org/10.1111/j.1529-8817.2009.00686.x

Ganesan, E.K., West, J.A. & Necchi, O. Jr. (2018) A catalogue and bibliography of non-marine (freshwater and estuarine) Rhodophyta (red algae) of India. *Phytotaxa* 1: 1–48. https://doi.org/10.11646/phytotaxa.364.1.1

Guiry, M.D. & Guiry, G.M. (2020) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org (accessed 03 March 2020).

Israelson, G. (1942) The freshwater Florideae of Sweden. Studies on their taxonomy, ecology and distribution. *Symbolae Botanicae Upsaliensis* 6: 1–134.

Jao, C.C. (1941) Studies on the freshwater algae of China VIII. A preliminary account of the Chinese freshwater Rhodophyceae.*Sinensia* 12: 245–290.

Johnston, E.T., Lim, P.-E., Buhari, N., Keil, E.J., Djawad, M.I. & Vis, M.L. (2014) Diversity of freshwater red algae (Rhodophyta) in Malaysia and Indonesia from morphological and molecular data. *Phycologia* 53: 329–341. https://doi.org/10.2216/13-223.1

Kumano, S. (1982) Four taxa of sections *Moniliformia, Hybrida* and *Setacea* of the genus *Batrachospermum* from temperate Japan. *Japanese Journal of Phycology* 30: 289–296.

Kumano, S. (2002) *Freshwater red algae of the world.* Biopress Ltd., Bristol, 375 pp.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and evolution* 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Kylin, H. (1912) Studien über die Schwedischen Arten der Gattungen *Batrachospermum* und *Sirodotia* nov. gen. *Nova Acata Reg. Soc. Sci. Upsal*. Sér 4, 3: 1–40.

Lam, D., Entwisle, T.J., Eloranta, P., Kwandras, J. & Vis, M.L. (2012) Circumscription of species in the genus *Sirodotia* (Batrachospermales, Rhodophyta) based on molecular and morphological data. *european Journal of Phycology* 47: 42–50. https://doi.org/10.1080/09670262.2011.645885

Necchi, O. Jr. (1990) *Revision of the genus Batrachospermum Roth (Rhodophyta, Batrachospermales) in Brazil.* Bibliotheca Phycologica 84. J. Cramer, Berlin, 201 pp.

Necchi, O. Jr. (1991) The Section *Sirodotia* of *Batrachospermum* (Rhodophyta, Batrachospermales) in Brazil. *Arch. Hydrobiol. Suppl. Algol. Stud***.** 62: 17–30.

Necchi, O. Jr., Sheath, R.G. & Cole, K.M. (1993) Distribution and systematics of the genus *Sirodotia* (Batrachospermales, Rhodophyta) in North America. *Journal of Phycology* 29: 236–243. https://doi.org/10.1111/j.0022-3646.1993.00236.x

Necchi, O. Jr., Vis, M.L. & Oliveira, M.C. (2007) Phylogenetic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North and South America. *Cryptogamie, Algologie* 27: 117–127.

Necchi, O. Jr., Agostinho, D.C. & Vis, M.L. (2018) Revision of *Batrachospermum* Section *Virescentia* (Batrachospermales, Rhodophyta) with the Establishment of the new genus, *Virescentia* stat. nov. *Cryptogamie, Algologie* 39: 313–338. https://doi.org/10.7872/crya/v39.iss3.2018.313

Necchi, O. Jr., Garcia, A. & Paiano, M. (2019) Revision of *Batrachospermum* sections *Acarposporophytum* and *Aristata* (Batrachospermales, Rhodophyta) with the establishment of the new genera *Acarposporophycos* and *Visia. Phytotaxa* 2: 51–65. https://doi.org/10.11646/phytotaxa.395.2.1

Paiano, M.O. & Necchi, O. Jr. (2013) Phylogeography of the freshwater red alga *Sirodotia* (Batrachospermales, Rhodophyta) in Brazil. *Phycological Research* 61: 249–255. https://doi.org/10.1111/pre.12027

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904. https://doi.org/10.1093/sysbio/syy032

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572– 1574. https://doi.org/10.1093/bioinformatics/btg180

Rossignolo, N.L. & Necchi, O. Jr. (2016) Revision of section *Setacea* of the genus *Batrachospermum* (Batrachospermales, Rhodophyta) with emphasis on specimens from Brazil. *Phycologia* 55: 337–346. https://doi.org/10.2216/15-144.1

Sheath, R.G. & Sherwood, A.R. (2002) Phylum Rhodophyta (Red Algae). *In:* John, D.M., Whitton, B.A. & Brook, A.J. (Eds) *An identification guide to freshwater and terrestrial algae*: *The freshwater algal flora of the British Isles.* Cambridge University Press, Cambridge, pp. 124–143.

Silvestro, D. & Michalak, I. (2012) RAxML GUI: a graphical front-end for RAxML. *Organism Diversity and evolution* 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Skuja, H. (1931) Untersuchungen über die Rhodophyceen des Süsswassers I-II. *Archiv für Protistenkunde* 74: 297–309.

Skuja, H. (1938) Die Süsswasserrhodophyceen der Deutschen Limnologischen Sunda-Expedition. *Archiv für Hydrobiologie* Supplement 15: 603–637.

Vis, M.L. & Sheath, R.G. (1999) A molecular investigation of the systematic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North America. *Phycologia* 38: 261–266. https://doi.org/10.2216/i0031-8884-38-4-261.1

2.7. SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

TABLE S1. Sequences of *rbc*L for *Sirodotia* species from Genbank used in the analysis (Fig. 2)

TABLE S2. Sequences of COI-5P for *Sirodotia* species obtained from Genbank used in the analyses (Fig 3)

TABLE S3. Morphological characters of *Sirodotia assamica* sp. nov. specimens examined in this study. Sample codes as in Table 2. Values for the morphometric data represent range and mean ± standard deviation (in parentheses). N= 15 except if otherwise indicated.

TABLE S4. Reproductive characters of *Sirodotia assamica* sp. nov. specimens examined in this study. Sample codes as in Table 2. Values for the morphometric data represent range and mean ± standard deviation (in parentheses). N= 15 except if otherwise indicated.

TABLE S1. Sequences of *rbc*L for *Sirodotia* species from Genbank used in the analysis (Fig.2).

Species	GenBank Accession number	Locality	Reference
S. aff. *huillensis*	JF344717	South Africa: X31-5 No. 1, Sabie River Catchment. Gerhard Strydom. iv.2000. (BHO A-1447, MICH).	Lam *et al*. (2012)
S. delicatula	KF557560	Malaysia: Selangor, Tributary of Gombak River in University of Malaysia Forest Reserve, unavailable coordinates, 27.viii.2018.	Johnston *et al*. (2014)
S. delicatula	AF126410	Costa Rica: Rt. 16 ~8km south of San Vito. 18.ii.1998.	Lam *et al*. (2012)
S. huillensis	AF126414	Mexico: Rt. 120 in Jalpar. 24.iv.1997.	Lam *et al*. (2012)
S. huillensis	JN408523	USA: Outlet canal of Montezuma Well, Arizona, 34º38'57" N, 111º45'08" W; T.A. Dempster. 24.iv.2011. (BHO A-0437, MICH).	Lam *et al*. (2012)
S. huillensis	JF344716	USA: Barton Spring, Austin, Texas, 30º15'50" N, 97º46'17" W; M.L. Vis & E. Johnston. 16.xii.2010. (BHO A-0410, MICH)	Lam *et al*. (2012)
Sirodotia sp.1[a]	DQ646474	Brazil: Minas Gerais, Serra do Cipó National Park, Alto do Palácio river, 100m from the highway MG-010. 19º17'00"S, 43º33'00"W, 25.vii.2003.	Necchi *et al*. (2007)
Sirodotia sp.1[a]	DQ646476	Brazil: São Paulo state, São José do Rio Preto, Macaco river, 20º50'31"S, 49º21'07"W, culture isolate.	Necchi *et al*. (2007)
Sirodotia sp.1[a]	KC951857	Brazil: São Paulo State, Guapiaçu, near the city, Córrego Cavenague, 20°47'14"S, 49°13'23"W; O. Necchi Jr., 02.vi.2008 (SJRP 31917).	Paiano & Necchi (2013)
Sirodotia sp.	KC951862	Brazil: Espírito Santo State, Domingos Martins, Pedra Azul, , "Rota do Lagarto" Road, 20°24'49"S, 41°02'23"W; O. Necchi Jr., 05.vii.2009 (SJRP 31920).	Paiano & Necchi (2013)
Sirodotia sp.	KC951865	Brazil: Goiás State, Alto Paraíso de Goiás, Chapada dos Veadeiros National Park, 14°09'58''S, 47°50'39''W; O. Necchi Jr., 06.iv.2010 (SJRP 31923).	Paiano & Necchi (2013)
Sirodotia sp.	KC951866	Brazil: Mato Grosso State, Rosana river, BR-163, between Sinop and Sorriso, 11°57'26''S, 55°31'01''W; O. Necchi Jr., 27.ix.2010 (SJRP 31924).	Paiano & Necchi (2013)
Sirodotia sp.	KC951867	Brazil: Mato Grosso State, Baixada Morena, Roquete River, Route BR-163, towards Santarém, 11º32'20''S, 55°31,14''W; O. Necchi Jr., 19.ix.2010; 10 m	Paiano & Necchi (2013)

Sirodotia sp.	KC951869	Brazil: Mato Grosso State, Sinop, BR-163, sentido Santarém; 11°36'04''S, 55°25'38'' W; O. Necchi Jr., 25.ix.2010 (SJRP 31925).	Paiano & Necchi (2013)
Sirodotia sp.	JF701687	U.S.A.: New Hampshire, near Enfield, 43°38′ N, 72°09′ W.	Necchi & Oliveira (2011)
S. suecica	AF029158	U.S.A.: Rhode Island, Chipuxet River at Rt. 138, West Kingston. 27.vi.1992	Vis *et al*. (1998)
S. suecica	AF126407	USA: Canada, 33 km west of Goose Bay on road to Churchill Falls, Labrador. 30. vii. 1993.	Vis & Sheath (1999)
S. suecica	AF209977	Australia: Nigger Creek, Herberton, Queensland; 17º23'00" S, 145º23'00" E; T. J. Entwisle (2430), 03.vii.1994 (MEL).	Vis & Entwisle (2000)
S. suecica	JF344715	Australia: Dom Dom Creek, Anderson Road crossing, ~1 km west of Naberthong, Victoria, 37º33'54" S, 145º39'08" E; T.J. Entwisle (3394), 05.i.2010 (NSW).	Lam *et al*. (2012)
S. suecica	JF344718	U.S.A.: Big Beaver Creek, at US 176 bridge crossing, Calhoun Co., South Carolina. E.K. Hollingsworth. 16.v.2008 (BHO, MICH).	Lam *et al*. (2012)
S. suecica	JF344719	New Zealand: Wanganui River, Beach track at the end of La Fontaine Road, 43º07'48" S, 170º36'37" E; M. L. Vis. 04.xii.2005 (BHO, MICH, WELT A027220, A027221).	Lam *et al*. (2012)
S. suecica	JF344721	Finland: Nytkymenjoki River, Hasakoski Rapid, (isolate 2) FI-10. P. Eloranta. 07.viii.2008 (MICH).	Lam *et al*. (2012)
S. suecica	JF344722	Finland: Kärkelänjoki River, Karjulankoski Rapid, (isolate 3) FI-18, 60º16'18" N, 23º35'49" E; P. Eloranta. 23.vi.2009. (MICH).	Lam *et al*. (2012)
S. suecica	JN408524	South Africa: Tributary of the Bot River off Van der Stel Pass Road near Cape Town; 34º09'31.5" S, 19º13'33" E; M.L. Vis, W.B. Chiasson & G. Ellis. 22.viii.2005. (BHO A-0264, MICH).	Lam *et al*. (2012)
S. tenuissima	JF344724	Finland: Tarhapäänkoski, Horhanvirta River, (isolate 5) FI-22, 62º20'29" N, 24º44'05" E; P. Eloranta. 16.vii.2010 (MICH).	Lam *et al*. (2012)
S. tenuissima	JF344725	Finland: Uittamonkoski Rapid, outlet of Lake Uittamonjärvi, (isolate 6) FI-23. P. Eloranta. 16.vii.2010. (MICH).	Lam *et al*. (2012)
S. tenuissima	AF126420	USA: Town Rd. in Coventry, Rhode Island. 18.iv. 1992.	Vis & Sheath. (1999)

a. Reported as *Sirodotia delicatula*.
Source: Author's own production

Tabela S2. Sequences of COI-5P for *Sirodotia* species obtained from Genbank used in the analyses (Fig 3).

Species	GenBank Accession number	Locality	Reference
S. huillensis	EU636739	U.S.A.: San Marcos, San Marcos river, Texas. M. L. Vis & R. G. Sheath. 1.xii.1993.	Sherwood *et al.* (2008)
S. suecica	EU636737	Canada: Labrador, un-named stream, 33 km west of Goose Bay on road to Churchill Falls. M. L. Vis & R. G. Sheath. 30.vii.1993.	Sherwood *et al.* (2008)
S. suecica	EU636738	South Africa: Tibutary of the bot river Vander Stel Pass, road near Cape Town.	Sherwood *et al.* (2008)
S. suecica	KM055337	U.S.A.: Big Beaver Creek, at US 176 bridge crossing, Calhoun Co., South Carolina. E.K. Hollingsworth. 16.v.2008 (BHO: A-0268, MICH).	Lam *et al*. (2012)
Sirodotia sp.	KF010483	Brazil: Espirito Santo state, Afonso Cláudio, Comunidade de Lajinha, near Rod. ES-165, 20°15'01"S, 41°03'43"W; O. Necchi Jr., 04.vii.2009 (SJRP 31922).	Paiano & Necchi (2013)
Sirodotia sp.	KF010486	Brazil: São Paulo State, Mirassol, River S. José dos Dourados, near fish pond "Aporé", 20º48'45"S, 49º34'29"W; 25.vi.2008 (SJRP 31918).	Paiano & Necchi (2013)
Sirodotia sp.	KF010487	Brazil: Espírito Santo State, Domingos Martins, Pedra Azul, "Rota do Lagarto" Road, 20º24'49"S, 41º02'23"W; 05.vii.2009 (SJRP 31920).	Paiano & Necchi (2013)
Sirodotia sp.	KF010488	Brazil: Goiás State, Alto Paraíso de Goiás, Chapada dos Veadeiros National Park, 14º09'58''S, 47º50'39''W; 06.iv.2010 (SJRP 31923).	Paiano & Necchi (2013)
Sirodotia sp.	KF010489	Brazil: Mato Grosso State, River Rosana, Route BR-163, between Sinop and Sorriso, 11º57'26''S, 55º31'01''W; 27.ix.2010 (SJRP 31924).	Paiano & Necchi (2013)
Sirodotia sp.	KF010490	Brazil: Mato Grosso State, Baixada Morena, Roquete River, Route BR-163, towards Santarém, 11º32'20''S, 55°31,14''W; 19.ix.2010.	Paiano & Necchi (2013)
Sirodotia sp.	KF010491	Brazil: Mato Grosso State, Sinop, BR-163, sentido Santarém; 11°36'04''S, 55°25'38'' W; 25.ix.2011 (SJRP 31925).	Paiano & Necchi (2013)

Source: Author's own production

TABLE S3. Morphological characters of *Sirodotia assamica* sp. nov. specimens examined in this study. Sample codes as in Table 2. Values for the morphometric data represent range and mean ± standard deviation (in parentheses). N= 15 except if otherwise indicated.

Sample	Sexuality	Whorl			Internodes	Primary fascicle				
		Shape	Diameter	Arrangement		Number of cells	Proximal		Distal	
							Length	Diameter	Length	Diameter
IN1a	Dioecious (male)	Pear-shaped, Obconical	400 – 523 (444.2 ± 40.2)	Dense	547 – 1177 (929.8 ± 217.2)	6 – 12 (8.4 ± 1.5)	Cylindrical, ellipsoidal 17 – 32 (31.5 ± 6.2)	Cylindrical, ellipsoidal 7 – 13 (8.5 ± 1.5)	Ellipsoidal, obovoidal, sub-shperical, 9 – 13 (10.9 ± 1.2)	Ellipsoidal, obovoidal, sub-spherical 5 – 9 (6.4 ± 0.8)
IN1b	Monoecious	Pear-shaped, Obconical	422 – 665 (561.3 ± 68.6)	Dense	612 – 949 (754.4 ± 101.7)	8 – 11 (9.8 ± 1.0)	Cylindrical, ellipsoidal 26 – 42 (30.4 ± 5.1)	Cylindrical, ellipsoidal 7 – 11 (8.7 ± 1.0)	Obovoidal 9,5 – 13 (11.2 ± 1.4)	Obovoidal 6 – 9 (7.0 ± 0.8)
IN2	Dioecious (male)	Pear-shaped, Obconical	412 – 599 (474.2 ± 55.8)	Loose	950 – 1088 (1015.6 ± 40.4)	7 – 9 (8.0 ± 0.7)	Cylindrical, ellipsoidal 22 – 28 (24.5 ± 2.0)	Cylindrical, ellipsoidal 8 – 10 (8.7 ± 0.5)	Obovoidal 12 – 14 (12.9 ± 0.8)	Obovoidal 6 – 8 (7.0 ± 0.6)

Source: Author's own production

Table S4. Reproductive characters of *Sirodotia assamica* sp. nov. specimens examined in this study. Sample codes as in Table 2. Values for the morphometric data represent range and mean ± standard deviation (in parentheses). N= 15 except if otherwise indicated.

Sample	Carpogonium				Carposporangia				Spermatangia
	Length	Diameter	Shape	Number of cells of carpogonial branches	Length	Diameter	Shape	Gonimoblast filament cell number	Diameter
IN1a	–	–	–	–	–	–	–	–	6 – 7 (6.4 ± 0.4)
IN1b	37 – 64 (51.7 ± 7,3)	10 – 16 (11.6 ± 1.4)	elongated cylindrical, ellipsoidal or lageniform	1 – 5 (-6)	11 – 14 (12.2 ± 0.8)	6 – 8 (7.2 ± 0.1)	Obovoidal	1 – 4	6 – 8 (7.1 ± 0.5)
IN2	–	–	–	–	–	–	–	–	6 – 8 (7.1 ± 0.7)

Source: Author's own production

3. CAPITULO 2

Revision of the genus *Sirodotia* (Batrachospermales, Rhodophyta) with description of four new species

Natalia L. ROSSIGNOLO[1], Morgan L. VIS[2], Monica O. PAIANO[3], Pertti ELORANTA[4], Janinna LEE[5], John A. WEST[6], E.K. GANESAN[7], Farishta YASMIN[8], Phaik-Eem LIM[9] & Orlando NECCHI Jr[1*].

[1] São Paulo State University, Zoology and Botany Department, Cristóvão Colombo, 2265, 15054-000 São José Rio Preto, São Paulo, Brazil; nataliarossignolo@yahoo.com.br

[2] Ohio University, Department of Environmental and Plant Biology, OH, USA; vis-chia@ohio.edu

[3] University of Hawai, School of Life Sciences, Honolulu, HI, USA; mpaiano@hawaii.edu

[4] University of Helsinki, Department of Biological and Environmental Sciences, Finland; pertti.eloranta@elisanet.fi

[5] Institute of Nature Conservation, Department of Freshwater Biology PAS Al. A. Mickiewicza 3, Poland; kwandrans@iop.krakow.pl

[6] School of Biosciences 2, University of Melbourne, Parkville VIC 3010, Australia; jwest@unimelb.edu.au

[7] Oceanographic Institute, Universidad de Oriente, Venezuela; ekganesan@gmail.com

[8] Nowgong College, Botany Department, Nagaon, 782001, Assam, India; farishtayasmin@gmail.com

[9] Institute of Ocean and Earth Sciences (IOES), University of Malaya, Malaysia; phaikeem@gmail.com

* Corresponding author: o.necchi@unesp.br

.

Running title: Revision of *Sirodotia* (Batrachospermales)

Artigo que será submetido à publicação na revista "Cryptogamie, Algologie",
http://sciencepress.mnhn.fr/en/periodiques/algologie

3.1. ABSTRACT

Genera of the freshwater red algal order Batrachospermales have been systematically revised using molecular and morphological data, but *Sirodotia* remains to be thoroughly reviewed. In this investigation, DNA sequence data for the *rbc*L, COI-5P and LSU markers for specimens collected worldwide were combined with morphological observations. Phylogenetic analyses showed the genus to be a monophyletic group with high support. Inter and intraspecific divergence values were well-delineated with higher interspecific (2.1-7% and 4.4-10.5%) and lower intraspecific (0-2.4% and 0-3.8%) variations for *rbc*L and COI-5P sequences, respectively. The LSU sequences revealed lower interspecific divergence values than the COI-5P sequences (0.7-3.3%) indicating lower resolution as a barcode marker. Nine species are recognized based on DNA sequence data, morphological characters and geographic distribution. Five species were previously described (*S. assamica*, *S. delicatula*, *S. huillensis*, *S. kennedyi* and *S. suecica*) and four new species are proposed (*S. amazonica*, *S. aquiloamericana S. cryptica*, and *S. delicatuliformis*). Diagnostic characters for the genus are confirmed to be asymmetric carpogonia with a basal protuberance and carposporophytes diffuse with indeterminant prostrate filaments producing determinate erect branches terminating in carposporangia. The following morphological characters were applied to distinguish species: primary fascicle cell number, spermatangial arrangement, origin of gonimoblast filament and size of carposporangia. Based on morphology, three species are proposed as synonyms of *S. suecica* (*S. sinica*, *S. segawae* and *S. yutakae*) and one species as synonyms of *S. delicatula* (*S. ateleia*). The status of three species (*S. cirrhosa*, *S. gardneri* and *S. huangshanensis*) could not be confirmed due to lack of type specimens or uninformative diagnostic characters.

Keywords: Batrachospermales, biogeography, COI-5P, freshwater Rhodophyta, LSU (28S), molecular systematics, *rbc*L, *Sirodotia*

3.2. INTRODUCTION

Over the past two decades, the systematics of the freshwater red algal order Batrachospermales has been extensively studied using molecular and morphological data (VIS *et al*., 1998; ENTWISLE *et al*., 2009). Recent revisional studies of sections within *Batrachospermum* have been conducted in order to rectify the paraphyly of this genus in relation to other genera of the order and resulting in most sections raised to generic rank (SALOMAKI *et al*., 2014; NECCHI *et al*., 2018, 2019; ROSSIGNOLO & NECCHI, 2016; VIS *et al*., 2020). Using combined morphological observations and DNA sequence data numerous new genera have been established within the order as follows: *Psilosiphon* (Entwisle, 1989), *Balliopsis* (SAUNDERS & NECCHI, 2002), *Petrohua* (VIS *et al*., 2007), *Kumanoa* (ENTWISLE *et al*., 2009), *Sheathia* (SALOMAKI *et al.,* 2014), *Nocturama* (ENTWISLE *et al*., 2016), *Torularia* (as *Setacea*, ROSSIGNOLO & NECCHI 2016 and Wynne 2019), *Lympha* (EVANS *et al*., 2017), *Volatus* (CHAPUIS *et al*., 2017), *Virescentia* (NECCHI *et al*., 2018), *Acarposporophycos* and *Visia* (NECCHI *et al*., 2019a)*, Montagnia* (NECCHI *et al*., 2019b) and *Paludicola* (VIS *et al*., 2020). Prior to the revison of *Batrachospermum* and the establishment of new genera, *Nothocladus*, *Sirodotia* and *Tuomeya* had long been recognized as unique based on morphological characters (KACZMARCZYK *et al*., 1992; NECCHI *et al*., 1993; SHEATH *et al*., 1996). The genus *Nothocladus* was recently investigated by Entwisle *et al*. (2016) and *Tuomeya* is monospecific, but *Sirodotia* has yet to be thoroughly reviewed.

The genus *Sirodotia* was proposed by Kylin (1912) and is similar in vegetative morphology to *Batrachospermum* and other genera with uniaxial, branched gametophyte thalli having whorls of fascicles from a main axis providing a beaded appearance (KUMANO, 2002). *Sirodotia* is distinguished by the presence of an asymmetrical carpogonium base (with a protuberance) and a diffuse carposporophyte extending along the main axis consisting of indeterminate, prostrate gonimoblast filaments with determinate erect branches producing carposporangia (KYLIN, 1912). It has been recognized as a distinct genus based on morphological data (NECCHI *et al*., 1993). As well, studies employing DNA sequence data have shown that specimens with morphological characters of *Sirodotia* are monophyletic (VIS & SHEATH, 1999; NECCHI et al., 2007; LAM *et al*., 2012; PAIANO & NECCHI, 2013; JOHNSTON *et al.,* 2014).

Skuja (1938a) listed 19 species of the genus *Sirodotia*, whereas Sheath (1984) catalogued 14 species. Kumano (2002) in his world monograph of freshwater red algae recognized eight species worldwide: *S. yutakae* Kumano, *S. segawae* Kumano*, S. sinica* Jao, *S. goebelii* Entwisle et Foard, *S. suecica* Kylin, *S. gardneri* Skuja ex Flint, *S. huillensis* (Welwitsch ex West et GS West) Skuja and *S. delicatula* Skuja. Five of these species are known from the type locality only or a few locations. Relevant general studies of freshwater Rhodophyta including species of *Sirodotia* have been conducted by Skuja (1931, 1938b), Jao (1941), Israelson (1942), Flint (1948, 1950), Reis (1969), Umezaki (1960), Sheath & Hymes (1980) and Entwisle & Kraft (1982). Kumano (1982) is the most comprehensive work on the taxonomy of this genus, in which six species were described and compared. Currently, there are 13 validly published and taxonomically accepted species (OTT, 2009; GUIRY & GUIRY, 2020) within the genus as follows: *Sirodotia assamica* Necchi, Rossignolo, Yasmin JA West & EK Ganesan, *Sirodotia cirrhosa* Skuja ex MS Balakrishnan & BB Chaugule, *Sirodotia delicatula* Skuja, *Sirodotia gardneri* Skuja ex Flint, *Sirodotia huangshanensis* ZX Shi & SL Xie, *Sirodotia huillensis* (Welwitsch ex West & GSWest) Skuja, *Sirodotia iyengarii* M.Baluswami & M.Babu, *Sirodotia kennedyi* AL Szinte, JC Taylor & ML Vis, *Sirodotia polygama* Skuja ex Flint, *Sirodotia segawae* Kumano, *Sirodotia sinica* CC Jao, *Sirodotia suecica* Kylin and *Sirodotia yutakae* Kumano.

For the species of *Sirodotia*, numerous morphological characters have been used for identification. Most commonly the morphology of the gametophyte, presence of spermatangia on specialized branches, the origin of the carpogonial branch (protuberant or non-protuberant side of the carpogonium base), and the size of the carpogonium and carposporangia have been considered important diagnoistic characters (NECCHI *et al*., 1993; KUMANO, 2002; NECCHI *et al*., 2007). However, overlap in morphometric characters among some species has been noted. Necchi (1991) reported that the length of the carpogonial branch and the shape and size of the carpogonia showed a wide and continuous variation in many populations of *S. delicatula* from Brazil, suggesting that these characters should not be used alone for the delimitation of species in the genus *Sirodotia*. Another character used is breeding system, Skuja (1938b) described *S. delicatula* as monoecious, while Umezaki (1960) and Kumano (1982) reported other populations as dioecious. Nevertheless, according to Umezaki (1960) sexuality is not considered important in distinguishing species of Batrachospermaceae because polygamy is frequent in the family. Thus, more

information about the diagnostic characters involving all species and representative populations is required.

Species of the genus have been reported from all continents, except Antarctica (NECCHI *et al*., 1993; ENTWISLE & FOARD, 1999; SHEATH & SHERWOOD, 2002; CARMONA *et al*., 2006; ELORANTA & KWANDRANS, 2007; NECCHI *et al*., 2007; ELORANTA *et al*., 2011). Necchi (1991) recognized only one species for Brazil (*S. delicatula*) with wide morphological variation and geographic distribution. Kumano (1982) described reproductive strucutures in six species from Japan and Malaysia and proposed two new species (*S. segawae* and *S. yutakae*), both containing specialized spermatangial branches. Necchi *et al*. (1993) analyzed 25 populations and ten type-specimens of *Sirodotia* and recognized the following species in North America: *S. huillensis*, *S. suecica* and *S. tenuissima*.

Few species have been recognized worldwide based on molecular and morphological data (NECCHI *et al*., 2007; VIS & SHEATH, 1999; LAM *et al*., 2012): *S. suecica* Kylin (temperate regions of North America and Europe), *S. delicatula* and *S. huillensis* (tropical and subtropical regions of the Americas and Asia). Molecular studies on *Sirodotia* provided insights on infra-generic taxonomy. Vis and Sheath (1999) analyzed *S. suecica*, *S. tenuissima* and *S. huillensis* from North America and proposed that *S. tenuissima* is synonym with *S. suecica*, whereas *S. huillensis* was kept as a distinct species. More recently, sequence data from *S. delicatula* from Brazil revealed it is morphologically and genetically distinct from *S. huillensis* and *S. suecica* (NECCHI *et al.,* 2007). Lam *et al.* (2012) suggested that the specimen *Sirodotia* aff. *huillensis* collected from South Africa could be a separate entity from *S. suecica*, *S. delicatula* and *S. huillensis*. In addition, molecular studies conducted with *Sirodotia delicatula* from Brazil (PAIANO & NECCHI, 2013) indicated the existence of cryptic *Sirodotia* species in Brazil. Johnston *et al*. (2014) showed based on DNA sequence data that *S. delicatula* from Malaysia and Brazil are not genetically similar. Considering the greater proximity to the type locality (Indonesia) of *S. delicatula*, they suggested that the Malaysian specimen represents *S. delicatula*, while the Brazilian species should be distinct (cryptic) species yet to be described.

Based on this set of information, it is evident that more DNA sequence data from a representative taxon sampling and geographic coverage is needed to evaluate species-level taxonomy and phylogenetic relationships of the genus *Sirodotia*. In addition, a reappraisal of morphological characters used for species delimitation is also

required. Therefore, the goals of this study were (1) to infer the phylogenetic relationships among species of the genus *Sirodotia* worldwide, based on molecular evidence of three genetic regions – the ribulose-1,5-bisphosphate carboxylase/oxygenase plastid gene (*rbc*L), the barcode region of the cytochrome oxidase subunit 1 mitochondrial gene (COI-5P), and the barcode region of the ribosomal DNA large subunit nuclear gene (LSU rDNA, 28S); (2) to pursue a morphological analysis to evaluate the characters used to circumscribe species within the genus.

3.3. MATERIAL AND METHODS

3.3.1. Morphological analyses

The type specimens of all species assigned to the genus *Sirodotia* were requested for morphological analysis. Those specimens were obtained as follows (herbarium acronyms according to Thiers *et al.,* 2020):

1. *Sirodotia angolensis* (West & GS West) Skuja, *Boletim da Sociedade Broteriana, Ser.* 2: 53, 1960. Isotype: Angola, Pungo Andongo, in rivulo de Cabondo socialis cum Podestemaceis, Welwitsch, ii. 1857 (LISU).
2. *Sirodotia fennica* Skuja, *Archiv für Protistenkunde* 74: 297, 1931. Lectotype: Finland, Somija, Karelia, H. Skuja, 13.vi.1930 (UPS).
3. *Sirodotia huillensis* (Welwitsch ex West & GS West) Skuja, *Archiv für Protistenkunde* 74:304, 1931. Isotype: Angola, Huílla, Lopollo, F. M. J. Welwitsch, v. 1860 (LISU).
4. *Sirodotia segawae* Kumano, *Botanical Magazine, Tokyo* 95:129, 1982. Isotype: Japan, Kyoto Sonobe, Ruri-kei, K. Hirayama, 17.iv.1966 (TNS-AL 169144).
5. *Sirodotia suecica* Kylin, *Nova Acta Regiae Soc. Sci. Upsal*. Ser 4, 3(3): 38, 1912. Isotype: Sweden, Skane, Osby, H. Kylin, 3.viii.1909 (UPS – A653877).
6. *Sirodotia yutakae* Kumano *Botanical Magazine, Tokyo* 95: 126, 1982. Isotype: Japan, Hyogo, Hojo, Ohara, S. Kumano, 2.iii.1961 (TNS-AL169145).

The type specimens of *S. cirrhosa* Skuja ex M.S.Balakrishnan & BB Chaugule, *S. huangshanensis* ZX Shi et SL Xie, *S. iyengarii* M. Baluswami & M. Babu and *S. sinica* CC Jao were requested, but they were not available for examination, so information from the protologues were used for morphological comparison. The type

specimens of *S. gardneri* Skuja ex Flint and *S. delicatula* Skuja could not be analyzed due to limited material. However, the morphometric data from the type specimens was available in Necchi *et al*. (1993).

In addition to the type specimens, we examined the morphology of *Sirodotia* samples with DNA sequence data, when specimens were available. These specimens were from numerous regions of the world including Africa, Asia, Australasia, Europe, North America and South America (Table S5). Some samples with molecular data could not be examined because there was no material for morphological analysis (*S. delicatula* CR1, *S. huillensis* MEX1, *S. suecica* FI3, FI4, FI5 and AU1, see Table S5). The specimens were examined for characters (size, arrangement and shape of the whorls, spermatangia and carposporangia; size, shape and number of cells of the primary fascicles; size and shape of the carpogonium and the origin of the gonimoblast filaments) currently used for species identification in the genus (NECCHI, 1991; NECCHI *et al*., 1993; VIS & SHEATH, 1999; KUMANO, 2002; NECCHI *et al*., 2007; LAM *et al.,* 2012).

For morphometric analyses, preliminary data from three samples (*Sirodotia* sp. BR3, BR4 and BR13, see Table S5) were used to determine the number of measurements needed. The following formula was applied to the data: $N = s/E\times$, where: s = sample standard deviation, E = standard error predetermined (in this case 0.05, minimum statistically valid value); x = sample mean (SOUTHWOOD, 1978). Using the preliminary data, we concluded that 15 measurements or counts for each characteristic per sample was sufficient; this number is similar to previous morphometric studies (NECCHI, 1990; AGOSTINHO & NECCHI, 2012; ROSSIGNOLO & NECCHI, 2016). For some samples, 15 measurements were not possible, especially for reproductive structures (e.g. carpogonium and carposporangium dimensions). Specimens were viewed using an Olympus SZH10 stereoscope and an Olympus BH-2 light microscope (Olympus Corp., Tokyo, Japan). Photomicrographs and measurements were taken with a Leica DFC 320 digital camera using LAS (Leica Application Suite) capture and image analysis software coupled to a Leica DM5000 microscope (Leica Microsystems, Wetzlar, Germany).

3.3.2. Molecular Analyses

Prior to DNA extraction, samples were ground with a Precellyst 24 tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). DNA extraction was conducted using the NucleoSpin plant II mini kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's protocol. Polymerase Chain Reactions (PCR) were performed with TopTaq™ Master Mix Kit (Qiagen GmbH, Hilden, Germany) and GoTaq G2 Hot Star Polymerase (Promega, Madison, USA), with a final volume of 25 µL containing 50 pmol genomic DNA and 5 pmol of each primer.

Regions of three genes were individually PCR amplified as follows: mitochondrial COI-5P barcode region (664 bp), chloroplast *rbc*L gene (1,282 bp) and nuclear LSU barcode region (~ 600 bp). For the COI-5P, the GazF1 and GazR1 primers and the thermocycler settings were used from Saunders (2005). The *rbc*L region was amplified in two overlapping fragments, using F160 with R897 and F650 with *rbc*LR (ABDELAHAD *et al*., 2015; ENTWISLE *et al*., 2016). The PCR conditions employed followed Vis *et al*. (1998) and Vis and Sheath (1999). The LSU barcode region was amplified using the primers T16N and T24U (~ 600 bp) and thermocycler settings provided in Saunders & Moore (2013). The PCR products were visualized using agarose gel electrophoresis in transilluminator and photodocumenter "Mini Bis Pro" and software "Gel CaptureTM (Micro Photonics, Allentown, USA). For samples with a single band, NucleoSpin Gel and PCR Clean-Up (Macherey-Nagel, Bethlehem, PA, USA) was used according to manufacturer's protocols. For the LSU barcode region, several bands were occasionally obtained. When several bands were observed for a single reaction, the entire PCR product was separated by DNA fragment size using gel electrophoresis, each band within the appropriate size range was excised from the gel using a new razor blade and purified similarily to the single band PCR products.

Purified PCR products were subjected to sequencing reactions using the ABI PRISM Big Dye Terminator kit. 3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Sequence data were obtained from the sense and antisense strands using the primers from the PCR reactions. The precipitated sequencing reaction products were sent for sequencing to Genomic Engenharia Molecular (http://www.genomic.com.br) on automated sequencer ABI PRISM™ 3100e. The individual sequence fragments of each gene were assembled and edited

using Geneious software version 7 (KEARSE *et al*., 2012) to produce a consensus sequence for each specimen.

The GenBank database, National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov; BENSON *et al*., 2013) was consulted for previously published *Sirodotia* sequences. All samples and associated information (species, location and GenBank number) were combined with information from the new sequences generated in this study (Tables S5-S7). For each gene region (*rbc*L, COI-5P and LSU), the new sequences (Table S5) and other sequences of *Sirodotia* and Batrachospermales (Tables S7) were aligned using the Geneious software version 7 (http: //www. geneious.com; KEARSE *et al*., 2012). All alignments were visually checked and edited as needed. The LSU marker (PCR products ~ 600 bp) contained two divergent domains for which the alignment was unclear, and these regions were excluded from the analyzes so that the final alignment was 547 bp. The concatenated alignment for *rbc*L, COI-5P and LSU was 2,943 bp. The resulting data set for a single gene consisted of 75 *rbc*L sequences, 34 COI-5P sequences and 29 LSU sequences, including 18 outgroup taxa. The set of concatenated data (*rbc*L, COI-5P and LSU) total 91 sequences consisting of 37 specimens with tree gene regions (*rbc*L, COI-5P and LSU), 35 specimens with two gene regions (32 specimens for *rbc*L and COI-5P; two specimens for *rbc*L and LSU; one specimen for COI-5P and LSU), 19 with one gene region (Tables S5-S7, Supporting information).

Distance trees for COI-5P and LSU markers were generated with the Geneious 7 software and its plug-ins. For the COI-5P marker, a distance analysis using Neighbor Joining (NJ) and TIM2+G model as determined by jModeltest 2.1.4 (DARRIBA *et al*., 2012) performed with Molecular Evolutionary Genetics Analysis (MEGA version X, KUMAR *et al*. 2018) with 10,000 bootstrap (bs) replicates. For the LSU marker, distance analysis using NJ and the best fit model HKY+G was performed with 10,000 bs replicates.

Phylogenetic inferences for the *rbc*L marker and concatenated dataset were conducted using Bayesian Inference (BI, MrBayes version 3.2.2; RONQUIST *et al*., 2012) and Maximum Likelihood Analysis (ML) (RaxML GUI version 8.2.4; STAMATAKIS, 2014). Phylogenetic analyzes were performed using the best fit model GTR+G+I model. Support values were determined for BI using 5,000,000 generations and for ML analyses using 10,000 bs replicates, saving one tree every 1000 generation. The first 500 trees were removed as burn-in prior to determining the

posterior probabilities. Burn-in cutoff was estimated in Tracer 1.7.1 (Rambaut *et al.* 2018).

The COI-5P sequences were analyzed for the existence of “barcode gaps”, namely, difference between interspecific and intraspecific variation (LELIAERT *et al*., 2014) using the single gene method “Automatic Barcode Gap Discovery” (ABGD; PUILLANDRE *et al*., 2012; http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html).

3.4. RESULTS

3.4.1. Molecular analyses

Seven *rbc*L, 20 COI-5P and 26 LSU sequences were newly generated for this study (Table S5). To complete the data set, 14 *rbc*L, three COI-5P and two LSU sequences were obtained from Genbank (Tables S6-S7). These sequences along with outgroup sequences were used for ML and BI analyses of the *rbc*L and concatenated dataset. BI and ML trees resulted in the same tree topology and only the ML trees were presented with support values for both analyses (Fig. S1).

All phylogenetic analyses of the concatenated and single gene data showed similar results with high BI and ML support values for the *Sirodotia* groups, except for minor variations in topology especially with specimens on longer branches. The concatenated analysis had *Sirodotia* sequences for all samples analyzed, including the LSU sequence of the *S. amazonica* from Brazil (BR21), for which it was not possible to obtain sequences for *rbc*L and COI-5P. The tree of concatenated data using ML analysis is shown, and the ML (*rbc*L) and NJ (COI-5P and LSU) trees for the single markers are provided as supplemental materials (Figs S1-S2, S4). The genus *Sirodotia* formed a well-supported clade (> 95 BS and > 0.95 PP) in all analyses (Fig. 5, Figs S1-S2, S4), showing that the genus *Sirodotia* represents a monophyletic group within a large clade of representative sequences for all genera of Batrachospermales (Fig. 5, Fig. S1). All clades of genera had high support values, but relationships among them were mostly poorly supported.

Within the genus *Sirodotia*, there were two major clades with high support values. One clade was composed of *S. suecica* sequences from numerous regions of the world - Africa, North America, Europe and Australasia and the other clade with sequences of all other species and from several continents - Africa, North America, South America and Asia. There were five clades of closely related specimens and

these were the previously described *S. assamica* and four that were determined as new species in this study, *S. amazonica*, *S. aquiloamericana*, *S. cryptica* and *S. delicatuliformis*. As well, there were individual sequences that were more distantly related to these clades and those were determined to be *S. delicatula, S. huillensis* and *S. kennedyi.* In the clade of *S. suecica*, a sample from South Africa (JN408524) was on a long branch with high support (> 95 BS and > 0.95 PP). The recently described species *S. kennedyi* was sister to a clade from South America, *S. delicatuliformis,* but the relationship was supported only by posterior probability in the concatenated analyses (PP=1.00) and in *rbc*L tree (PP=0.93). Interspecific divergences were high among all clades (2.3-7.0%; 4.4-10.5%), whereas intraspecific variation was low (0-2.1%; 0-2.4%) for *rbc*L and COI-5P, respectively.

The *rbc*L tree was mostly congruent with the concatenated tree and exhibited two major clades, one with *S. suecica* and the other with all other species. The one clade containing the sequences of *S. suecica* showed a wide intraspecific variation (0.1-2.4%). The other large clade had eight smaller clades, each representing one species (Fig. S1). The first clade contained sequences of *S. delicatula* from Malaysia (high support BS > 95%, PP> 0.90) with a recently described species (*S. assamica* from India, intraspecific divergence 0.4%). The second clade with high support includes a sequence previously referred as *S.* aff. *huillensis* from Africa (here interpreted as the true *S. huillensis)* that diverged from the closest species *S. amazonica* and *S. cryptica* in 2.1-3.2%. The third clade had medium to high support (79% BS, 1.00 PP) with samples of *S. cryptica* from Midwestern Brazil (states of Goiás and Mato Grosso,) with a variation of 0.2-0.8%. The fourth clade (full support) contained four samples of *S. amazonica* from Brazil (states of Mato Grosso and Roraima, divergence of 0-0.3%). The fifth clade (BS > 95%; PP > 0.90) is represented by samples of *Sirodotia* from North America previously referred as *S. huillensis* (divergence 0-0.5%), here proposed as a new species (*S. aquiloamericana*). The sixth clade (79% BS, 0.93 PP) includes one sequence with a recently described species from Africa (*S. kennedyi*), that diverged from the neighbor species *S. delicatuliformis* by 1.6-1.9%. The seventh clade is represented by *Sirodotia* sequences from southeastern Brazil and a sample from Costa Rica (AF126410) referred to as *S. delicatula*. The divergence among the sequences of this clade was 0-0.7%, and this clade is here interpreted as a new species (*S. delicatuliformis*). In summary, the phylogenetic analysis of the *rbc*L marker showed nine species of *Sirodotia*, of which

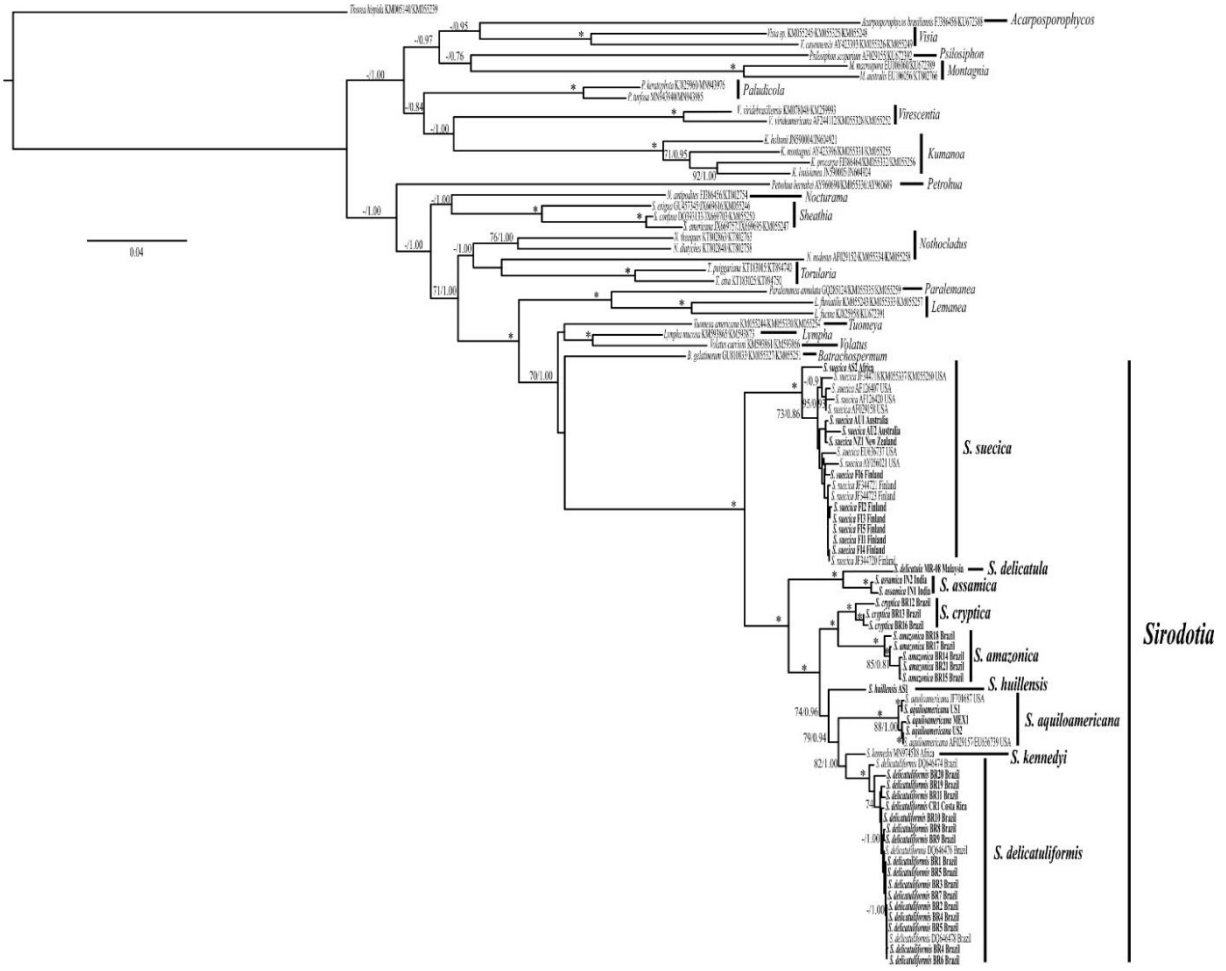
five have already been described (*S. assamica*, *S. delicatula*, *S. huillensis*, *S. kennedyi* and *S. suecica*) and four species that are being described here (*S. amazonica*, *S. aquiloamericana*, *S. cryptica* and *S. delicatuliformis*).

The COI-5P NJ tree (Fig. S2) with twenty new COI-5P sequences generated in this study combined with seventeen GenBank *Sirodotia* sequences showed well-supported groups representing eight species. COI-5P sequence was not available for *S. kennedyi*. As shown by *rbc*L and concatenated analysis, sequences of each group represented distinct species, as follows: *S. suecica*, *S. delicatula*, *S. assamica*, *S. huillensis*, *S. aquiloamericana*, *S. delicatuliformis*, *S. amazonica* and *S. cryptica*. The clade containing the sequences of *S. suecica* showed high support (BS> 95%) and high intraspecific variation (0-3.8%), which was due to a sample from Africa (EU636738) that diverged by 2.4-3.8% with the other species of *S. suecica* (variation of 0-2.6%). This divergent sequence possibly represents a new species. The larger group that contained seven species exhibited high interspecific divergences (≥ 4.2%) and low divergence within groups (≤ 2.5%). Species delineation analysis using ABGD for the COI-5P barcode region (Fig. S3) with 38 sequences showed partitioning into eight groups, which were consistent with the concatenated data set and the single gene trees (Figs S1-S4).

The LSU NJ tree (Fig. S4) for the 26 new LSU sequences and two sequences from GB showed similar results as the other single gene analyses (*rbc*L and COI-5P). The NJ tree showed the genus *Sirodotia* with two distinct groups. The first group, with high support (BS = 95%), represents the samples of *S. suecica* with divergence of 0-0.4%. The second group with medium support (BS = 88%) showed several smaller groups with medium to high support (BS = 82-99%) containing five species: *S. amazonica, S. assamica, S. cryptica, S. delicatula* and *S. delicatuliformis*. The divergence observed among the groups were 0.7-3.3% and the divergence within groups were lower (0-0.7%). However, the divergences (0.7-3.3%) and support values (BS<95) among the *Sirodotia* groups were lower than those of COI-5P sequences (BS>95 and 4.4-10.5%). Despite the lower values, the tree showed six species for which LSU sequences were obtained: *S. amazonica, S. assamica*, *S. cryptica*, *S. delicatula*, *S. delicatuliformis*, and *S. suecica*.

Fig. 5. Phylogenetic tree (ML) showing the relationship of species within Sirodotia and genera of the Batrachospermales and Thoreales based on rbcL, COI-5P and LSU

concatenated sequence data. * = bootstrap (bs) > 95 and posterior probability (pp) >0.95 support; nodes without values indicate <70 bs and < 0.80 pp. Specimen code as in Table S1. Some sequences represent more than one collection with identical sequence, the number of additional sequences is shown in parentheses and this information is noted in Table S5. Scale represents substitutions per site.



Source: Author's own production

3.4.2. Morphological analyses

Among the 40 samples of *Sirodotia* available for molecular data (Table S5), 25 had sufficient specimens for morphological analysis. The samples and type specimens showed some consistent features for vegetative and reproductive characters for the genus, as well as for species delimitation (Figs. 6-52, Tables S8-S10). Two characters common to all specimens were the obconic or pear-shaped whorls (Figs. 6, 11, 16, 22, 27, 32, 39, 45 and 48) and the carpogonia with a hemispherical protuberance in the basal portion. When carposporophytes were observed, they consisted of gonimoblast filaments prostrate and indeterminate, producing short erect branches with carposporangia at the tips. Some characters were noted to consistently differ among

specimens and have proved to be applicable to distinguish species within the genus. In particular, the number of primary fascicle cells, the occurrence of spermatangia arranged in clusters, the origin of gonimoblast filaments in relation to the carpogonia (on the same or opposite side of the basal protuberance) and the size of carposporangia were shown to be good diagnostic criteria.

3.4.2.1. Taxonomic treatment

Sirodotia Kylin, Nova Acta Regiae Soc. Sci. Upsal. Ser. IV, 3: 38 (1912).

Synonym: Section *Sirodotia* **(**Kylin) Necchi & Entwisle, Phycologia 29, 4: 485 (1990).

Type species: *Sirodotia suecica* Kylin, Nova Acta Regiae Soc. Sci. Upsal. Ser. IV, 3: 38 (1912).

Revised description: Plants monoecious, dioecious or polyoecious, bluish green to yellowish green; branching irregular; whorls well-developed or reduced, contiguous or separated, obconic or pear-shaped; cortical filaments of the main axis well-developed, one or two layers; primary fascicles with cells variable in shape, cylindrical, ellipsoidal, obovoid, subspherical or spherical; secondary fascicles abundant, covering the entire or two-thirds of internode, equal to or less than the length of the primary fascicles; spermatangia spherical, subspherical or obovoid on primary or secondary fascicles; carpogonial branches well-differentiated from the fascicles, straight, rarely curved, developing from the periaxial, proximal, median and distal cells of primary fascicles or cortical filaments, rarely on the secondary fascicles, short, composed of disc- or barrel-shaped cells; involucral filaments few and short, composed 1-4 cylindrical or ellipsoidal cells; carpogonia asymmetrical, with a hemispherical protuberance in the basal portion; trichogynes sessile and cylindrical, cylindrical-elongated, conical-elongated, clavate, fusiform, lageniform or ellipsoidal, with or without wavy margins; carposporophyte diffuse extending along the internode; gonimoblast filaments develop on the same side or opposite side of the basal protuberance of the carpogonium; gonimoblast filaments prostrate and indeterminate, composed of 1-5 cylindrical cells, producing short, erect branches, formed by cylindrical or ellipsoidal cells, with terminal or sub-terminal carposporangia; carposporangia large or small, obovoidal, ellipsoidal or subspherical.

Diagnostic characters: the genus is characterized by diffuse carposporophytes composed of prostrate gonimoblast filaments producing short, erect filaments with terminal carposporangia; and asymmetric carpogonium, with a semi-spherical basal protuberance; the shape of the whorls (obconic or pear-shaped) can be used as a

complementary character, however it is not exclusive to the genus and can be observed in species of *Kumanoa* (NECCHI & VIS, 2012) and *Paludicola* (VIS *et al*., 2020).

Distribution: the genus has been collected in temperate, tropical, subtropical and sub-polar regions of North and South America, Africa, Asia, Australasia and Europe.

Key to the species of the genus *Sirodotia*

1a. Carposporangia small, 8-10 µm in length, known distribution restricted to Africa (Angola, South Africa and Zambia)…….....…….....2

1b. Carposporangia large, 10-21 µm in length, known distribution in Africa, Americas, Asia, Australasia and Europe…….....3

2a. Primary fascicle 3-5 cells …….....*S. kennedyi*

2b. Primary fascicle 5-9(-10) cells …….....*S. huillensis*

3a. Spermatangia arranged in clusters….....*S. assamica*

3b. Spermatangia isolated or in pairs.....4

4a. Gonimoblast initial developing from the non-protuberant side of the carpogonium……..…….....*S. suecica*

4b. Gonimoblast initial developing from the protuberant side of the carpogonium.....5

5a. Known distribution restricted to Asia (Indonesia, Japan and Malaysia)……...*S. delicatula*

5b. Known distribution in the Americas….....6

6a. Carposporangia wide, 8-13 µm in diameter…….....*Sirodotia amazonica* and *Sirodotia cryptica*

6b. Carposporangia narrow, 6-8.5 (-9.5) µm in diameter.....7

7a. Known distribution in southern North America (Costa Rica) and South America (Brazil)……..…….*Sirodotia delicatuliformis*

7b. Known distribution in arid regions of North America (USA, Mexico)……...……..*Sirodotia aquiloamericana*

3.4.2.2. Species description

Sirodotia amazonica Necchi, Rossignolo & Paiano **sp. nov.** Figs. 6-10.

Holotype: SJRP 31924, O. Necchi Jr., 27.ix.2010.

Type locality: Brazil, Mato Grosso, River Rosana, Route BR-163, between Sinop and Sorriso; 11º57'26" S, 55º31'01" W.

Description. Plants monoecious or dioecious; whorls 249-491 µm in diameter; primary fascicles, 4-8 cells; proximal cells cylindrical or ellipsoidal; distal cells spherical, subspherical, obovoidal or ellipsoidal; secondary fascicles abundant, covering the entire internode; spermatangia spherical or obovoidal, 1-3 in a group, few or abundant on primary or secondary fascicles, 6-9 µm in diameter; carpogonial branches composed of 1-4 disc- or barrel shaped cells, arising from periaxial or proximal cells of primary fascicles, rarely on the secondary fascicles or cortical filaments, short, 8.5-25 µm in length; carpogonia with sessile, elongate cylindrical (with wave margins) or fusiform trichogynes, 35-58(-62) µm in length, 8-14(-15) µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments prostrate with erect branches of 1-4 cells; carposporangia obovoidal or subspherical, 10-18(-19) µm in length, (7-)8-13 µm in diameter.

Additional specimens examined: SJRP 32139, SJRP 32140, SJRP 32576, SJRP 32577, and SJRP 32578 (Table S5).

Etymology: the species epithet indicates that the alga occurs in the Amazonian region in Brazil.

Distribution: South America – Brazil (mid-western and northern Brazil).

Representative DNA sequences: COI-5P (KF010489, KF010490, BR17), *rbc*L (KC951866, KC951867, BR17) and LSU (BR14, BR15, BR17).

Remarks: The phylogeography study by Paiano *et al.* (2013) showed the existence of two cryptic species in Brazil. Since no morphological characteristics were observed to distinguish them, but a high interspecific divergence (2.3-3.0% for *rbc*L and 4.4-6.2% COI-5P). We conclude they are distinct. *Sirodotia amazonica* is very similar to two species found in Brazil, *S. delicatuliformis* and *S. cryptica*, overlapping for most morphometric and morphological characters. However, *S. amazonica* is distinguishable from *S. delicatuliformis* based on the wider carposporangia (8-13 versus 6-8.5(-9.5) µm in diameter) and is highly divergent in sequence data (3.4-3.8% for *rbc*L and 7.1-8.9% for COI-5P). *S. amazonica* and *S. cryptica* are cryptic species

genetically distinct from each other with high divergence (2.3-3.0% for *rbc*L and 4.4-6.2% for COI-5P).

Sirodotia aquiloamericana Vis, Necchi & Rossignolo **sp. nov**. Figs. 11-15.

Holotype: BHO-0437. T.A. Dempster, 24.iv.2011.

Type locality: USA, Arizona, outlet canal of Montezuma; 34º38'57" N, 111º45'08" W.

Description. Plants dioecious; whorls 408-675 µm in diameter; primary fascicles 7-12 cells; proximal cells cylindrical or ellipsoidal; distal cells subspherical, ellipsoidal or obovoidal; secondary fascicles abundant, covering the entire internode; spermatangia spherical or obovoidal, 1-3, few or abundant on primary or secondary fascicles, 5-7(-8) µm in diameter; carpogonial branches composed of 1-3 disc- or barrel shaped cells, arising from periaxial or proximal cells, short, 11-14 µm long; carpogonia with sessile, elongate cylindrical (with wavy margins) or fusiform trichogynes, 29-42 µm in length, 6-7.5 µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments with erect branches of 2-5 cells; carposporangia obovoidal, 10-13 µm in length, 6-8 µm in diameter.

Additional specimens examined: BHO A-0410, MEX1 (Table S5).

Etymology: the species epithet indicates that the alga is known from North America.

Distribution: North America – Mexico and the United States (Arizona and Texas).

Representative DNA sequences: COI-5P (US1, US2, EU636739) and *rbc*L (JN408523, JF344716, AF126414)

Remarks: this species has been reported as *S. huillensis* (NECCHI *et al*.,1993; VIS & SHEATH, 1999; LAM *et. al*., 2012) from North America. It has been described as having spermatangia arranged in clusters. However, this arrangement was not confirmed in this species and we have observed only abundant spermantangia in some specimens. The true arrangement in clusters in *Sirodotia* was observed only in *S. assamica*. *S. aquiloamericana* specimens are genetically divergent from those of *S. huillensis* from Africa (interspecific divergence of 2.7-2.8% for *rbc*L; 6.8% for COI-5P). Thus, the North American material represents a distinct species that is here described. This species is most closely comparable to *S. delicatuliformis* based on the narrow carposporangia, 6-8.5 (-9.5) µm in diameter. However, *S. aquiloamericana* differs from *S. delicatuliformis* in having wider whorls (408-675 versus 169-491 µm in diameter) and geographical distribution (arid regions of North America versus southern North

American and South America). In addition, they are genetically divergent (2.4-2.7% for *rbc*L and 6.6-7.7% for COI-5P).

Sirodotia assamica Necchi, Rossignolo, Yasmin, West & Ganesan, *Phytotaxa* 437: 125 (2020). Figs. 16-21.

Type: SJRP 32584. F. Yasmin, 25.ii.2019 (Holotype).

Type locality: India, Assam, Nagaon District, Chapanalla; 26º19’13.7” N, 92º10’16.5” E.

Description. Plants dioecious or monoecious; whorls 400-665 µm in diameter; primary fascicles, 6–11(-12) cells; proximal cells cylindrical or ellipsoidal; distal cells obovoidal or ellipsoidal; secondary fascicles abundant, covering the entire internode; spermatangia spherical, arranged in clusters on primary or secondary fascicles, 6-8 µm in diameter; carpogonial branches straight or slightly curved, short, composed of 1-5(-6) disc- or barrel shaped cells; arising from periaxial cells of primary fascicles, 7-23 µm in length; carpogonia with sessile, elongate cylindrical, ellipsoidal or lageniform trichogynes, 37-64 µm in length, 10-14(-16) µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments with erect branches of 1-4 cells; carposporangia obovoidal, 11-14 µm in length, 6-8 µm in diameter.

Additional specimens examined: SJRP 32583, SJRP 32585 (Table S5).

Distribution: Asia – India (northeastern).

Representative DNA sequences: COI-5P (MN508239, MN508240) and *rbc*L (MN496129, MN496130).

Remarks: *Sirodotia assamica* most distinguishing feature is the occurrence of spermatangia arranged in clusters, thus far not confirmed for any other species of *Sirodotia.* It is most closely comparable to *S. delicatula* based on the vegetative and reproductive characteristics and the occurrence in or near India (Table S6). *Sirodotia assamica* differs from *S. delicatula* in having spermatangia in clusters, larger whorls (400–665 µm versus 137–433 µm in diameter), distal fascicles cells ellipsoidal or obovoid (L/D 1.3-2.1) in *S. assamica* and subspherical or obovoid (L/D 1.1-1.7) in *S. delicatula* and the known geographic distribution restricted to northeastern India. Moreover, they are genetically quite divergent (2.5–2.7% for *rbc*L and 4.2%–4.7% for COI-5P).

Sirodotia cryptica Necchi, Rossignolo & Paiano **sp. nov.** Figs. 22-26.

Type: SJRP 32575, F.R. Borges, 15.iv.2014.

Type locality: Brazil, Goiás, Highway GO-070, between the municipalities of Jussara and Itaberaí; 16º00'32.7" S, 48º55'55.9" W.

Description. Plants monoecious or dioecious; whorls 223-559 µm in diameter; primary fascicles (4-)5-10(-11) cells; proximal cells cylindrical or ellipsoidal; distal cells spherical, ellipsoidal or obovoidal; secondary fascicles abundant, covering the entire internode; spermatangia spherical or obovoidal, 1-3 in a group, few or abundant on primary or secondary fascicles, 6–9 µm in diameter; carpogonial branches composed of 1-4 disc- or barrel shaped cells, arising from periaxial, proximal or median cells of primary fascicles, rarely on the secondary fascicles or cortical filaments, short, 10-25 µm long; carpogonia with sessile, elongate cylindrical (with wavy margins), elongate-conical or fusiform trichogynes, sometimes with anomalous shapes (bent end), (24-) 26-58 µm in length, 9-13(-15) µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments prostrate with erect branches of 2-5 cells; carposporangia subspherical, ellipsoidal or obovoidal, 10-17 µm in length, (7-)8-11 µm in diameter.

Additional specimens examined: SJRP 31923 and SJRP 31925 (Table S5).

Etymology: the species epithet indicates that the alga is cryptic with another species described in this study (*S. amazonica*).

Distribution: South America – Brazil (midwestern)

Representative DNA sequences: COI-5P (KF010488, BR13, KF010491), *rbc*L (KC951865, BR13, KC951869) and LSU (BR12, BR13, BR16).

Remarks: this species is morphologically indistinguishable from another species occurring in Brazil described in this study (*S. amazonica*) but genetically divergence (2.3-3.0% for *rbc*L and 4.4-6.2% for COI-5P)**.** Thus, it is here described as a new species.

Sirodotia delicatula Skuja, *Archiv für Hydrobiologie Supplement* 15: 614 (1938). Figs. 27-31.

Homotypic synonym: *Batrachospermum delicatulum* (Skuja) Necchi & Entwisle, *Phycologia* 29: 486 (1990).

Heterotypic synonym: *Sirodotia ateleia* Skuja, ***Archiv für Hydrobiologie Supplement* 15: 617 (1938).**

Type: UPS A-003747, D.L. Sunda-Expedition, 19.ix.1928 (Lectotype).

Type locality: Indonesia, Java Island, Bogor, Tijiliwong; 6º35’21” S, 106º48’19” E.

Revised description. Plants monoecious or dioecious; whorls 137-484 µm in diameter; primary fascicles, 5-10 cells; proximal cells cylindrical or ellipsoidal; distal cells subspherical or obovoidal; secondary fascicles abundant, covering the entire internode; spermatangia spherical or obovoidal, 1-3, few or abundant on primary fascicles, (4-)7-8 µm in diameter; carpogonial branches composed of 2–5(-7) disc- or barrel shaped cells, arising from the periaxial or middle cells of the primary fascicles, short, 8-17(-20) µm in length; carpogonia with sessile, elongate cylindrical (with wavy margins), fusiform, ellipsoidal or clavate trichogynes, (19-)24-70 µm in length, 5-13 µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments with erect branches of 2-4 cells; carposporangia obovoidal, (8-)10-16 µm in length, 5-10 µm in diameter.

Additional specimens examined: BHO A-0984 (Table S5).

Distribution: Asia – Indonesia, Japan and Malaysia.

Representative DNA sequences: COI-5P (MR-08), *rbc*L (KF557560) and LSU (MR-08).

Remarks: this species is morphologically similar to four species of *Sirodotia* (*S. delicatuliformis*, *S. amazonica*, *S. cryptica* and *S. aquiloamericana*) based on large carposporangia, spermatangia single or in pairs on primary fascicles and gonimoblast initial developing from the protuberant side of carpogonium. It is distinguishable from those species based on the geographic distribution restricted to Asia versus Americas. *Sirodotia delicatula* has been recorded from Indonesia and Japan and more recently reported from Malaysia including *rbc*L sequences (JOHNSTON *et al*., 2014). It had been previously reported in South America (Brazil) (NECCHI, 1991; NECCHI *et al*., 1993, 2007; PAIANO & NECCHI, 2013). However, Asian specimens are distinguishable from South American ones based on the divergences in the DNA sequences (4.4%-5.0% for *rbc*L and 8.6-10.1% for COI-5P) and in the known geographic distribution (Asia versus South America). Since Malaysia is closer to the type locality, the Asian specimens are regarded as representing the species. *Sirodotia ateleia* Skuja was considered synonym of *S. delicatula* by Umezaki (1960) and Necchi (1991) because of the combined characters. On the other hand, based on the examination of North America populations identified as *S. huillensis* and the type of *S. ateleia*, Necchi *et al.* (1993) considered these two taxa to be synonymous by the size

and shape of the whorls. In this study we followed the proposal by Umezaki (1960) and Necchi (1991) of *S. ateleia* be synonymized with S. delicatula based on similarity of morphological characters and geographic distribution in Asia, although *S. ateleia* has longer carpogonia (42-70 µm versus (19-)24-57 µm in length).

Sirodotia delicatuliformis Necchi, Rossignolo & Paiano **sp. nov.** Figs. 32-38.

Holotype: SJRP 31918. O. Necchi Jr., 25.vi.2008.

Type locality: Brazil, São Paulo State, Mirassol, São José dos Dourados River; 20º48'45" S, 49º34'29" W.

Description. Plants monoecious, dioecious or polyoecious; whorls 169-491 µm in diameter; primary fascicles 5-10(-13) cells; proximal cells cylindrical, ellipsoidal or obovoidal; distal cells obovoidal, ellipsoidal or spherical; secondary fascicles abundant, covering the entire internode; spermatangia spherical or obovoidal, single or in pairs on primary or secondary fascicles, 5-8(-8.5) µm in diameter; carpogonial branches composed of 0-4 disc- or barrel shaped cells, arising from periaxial, proximal or distal cells of primary fascicles, rarely on secondary fascicles or cortical filaments, short, 6-22 µm in length; carpogonia with sessile, elongate cylindrical (with wavy margins) or fusiform trichogynes, sometimes with anomalous shapes (bifurcated or with bent end), (20-)22-55(-59) µm in length, 8-14(-16) µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments with erect branches of 1-4 cells; carposporangia obovoidal or ellipsoidal, 11-16 µm in length, 6-8.5(-9.5) µm in diameter.

Additional specimens examined: SJRP 23450, SJRP 23501, SJRP 31915, SJRP 31916, SJRP 31917, SJRP 31919, SJRP 31920, SJRP 31921, SJRP 31922, SJRP 32156, SJRP 32579 and SJRP 32580 (Table S5).

Etymology: the species epithet indicates that the alga is morphologically very similar to *S. delicatula*.

Distribution: South America – Brazil (southeastern) and North America – Costa Rica.

Representative DNA sequences: COI-5P (KF010483, KF010486 e KF010487), *rbc*L (KC951857, KC951858, KC951863) and LSU (BR3, BR4, BR9).

Remarks: this species has been previously reported as *S. delicatula* (Necchi 1991; Necchi *et. al* 1993; 2007) from South and North America. Based on recent studies including molecular data (PAIANO & NECCHI, 2013; JOHNSTON *et al*., 2014; this study), the sequences from South and North America were showed to be genetically

divergent from the sequence of Malaysia of *S. delicatula*. Thus, it represents a distinct species that is here described. The species is highly divergent from the Malaysian *S. delicatula* in sequence data of *rbc*L (4.4-4.7%) and COI-5P (8.7-9.8%). However, these two species are morphologically very similar with considerable overlap for all morphological characters but the disjunct geographic distribution that can be applied as criterion to distinguish them. In addition, this species formed a clade in the tree other species (*S. amazonica*, *S. cryptica* and *S. aquiloamericana*) in both the *rbc*L and COI-5P trees with high interspecific variation, 2.2-3.7% for *rbc*L and 6.1-8.9% for COI-5P. The morphology among these species is similar, but *S. delicatuliformis* is distinguishable from *S. amazonica* and *S. cryptica* based on the narrow carposporangia (6-8.5(-9.5) versus 8-13 µm in diameter), and from *S. aquiloamericana* by having smaller whorls (169-491 versus 408-675 µm in diameter).

Sirodotia huillensis (Welwitsch ex West & G.S.West) Skuja, *Archiv für Protistenkunde* 74: 304 (1931). Figs. 39-44.

Basionym: *Batrachospermum huillense* Welwitsch ex West & G.S.West, *Journal of Botany* 35: 3 (1897).

Type: BM 001043858, F. M. J. Welwitsch, v.1860 (Holotype); LISU (Isotype).

Type locality: Africa, Angola, Huila, Lopollo**,** 14º47’51” S, 14º40’03” E.

Revised description: Plants monoecious or dioecious; whorls 162-364 µm in diameter; primary fascicles 5-9(-10) cells; proximal cells cylindrical, ellipsoidal or obovoidal; distal cells subspherical, obovoidal or ellipsoidal; secondary fascicles abundant, covering the entire internode; spermatangia spherical, 1-3, few or abundant on primary fascicles, 5-7 µm in diameter; carpogonial branches composed of 1-3 disc- or barrel shaped cells, arising from periaxial or distal cells of primary fascicles, short, 5-14 µm in length; carpogonia with sessile, elongate cylindrical (with wavy margins), ellipsoidal, fusiform or lageniform trichogynes, 28.5-48 µm in length, 5.5-12 µm in diameter; gonimoblast initial developing from the protuberant side of the carpogonium; gonimoblast filaments with erect branches of 2-4 cells; carposporangia obovoidal, 8–10 µm in length, 5–8 µm in diameter.

Additional specimen examined: BHO A-1447 (Table S5).

Distribution: Africa – Angola, South Africa.

Representative DNA sequences: COI-5P (AS1) and *rbc*L (JF344717).

Remarks: this species is most closely comparable to *S. kennedyi* based on the reduced whorls (162-364 µm in diameter), shorter carposporangia (8-10 um in length) and geographic distribution (occurrence in Africa). However, *S. huillensis* differs from *S. kennedyi* in having a greater number of cells in primary fascicle (5-9(-10) versus 3-5), a small number of cells in the carpogonial branches (1-3 versus 3-4), a shorter carpogonial branch (5-14 versus 16-22 µm in length) and a greater number of cells in the erect gonimoblast filament (2-4 versus 1-2, Tables S4-S6). In addition, they are genetically divergent (2.1% for *rbc*L). This species was described from Africa by Skuja (1931) and from North America (Arizona and Texas) by Necchi *et. al* (1993) as having spermatangia arranged in clusters. However, this type of arrangement was not confirmed in this species based on the analysis of the type and protologue. The true arrangement in clusters was observed only in *S. assamica*. The species referred by Lam *et. al* (2012) as *S.* aff. *huillensis* (BHO A-1447), which was analyzed in this study, could not be conclusively identified based on morphology for the limited morphological characters provided or examined. Phylogenetic analyses based on molecular data (LAM *et al*, 2012; this study) of *S.* aff. *huillensis* collected in South Africa was inferred as a sister clade of the species described as *S. huillensis* in North America (interspecific divergence of 2.7-2.8% for *rbc*L; 6.8% for COI-5P). Therefore, considering this information and that the specimen of *S.* aff. *huillensis* was collected closer to type locality of *S. huillensis*, we interpreted that it should be associated with *S. huillensis* and the North American specimens represent another species to be described.

Sirodotia kennedyi A.L.Szinte, J.C.Taylor & M.L.Vis, *Phycologia* (2020). Figs. 45-47.
Type: SANDC 19-566, Coll. M.P. Kennedy, 07.vii.2011 (Holotype); BHO A-0946 (Isotype).
Type locality: Zambia, Mutinondo River, Mutinondo Wilderness, 12º16.101’ S, 31º22.869’ E.
Description: Plants dioecious; whorls 198-315 µm in diameter; primary fascicles, 3-5 cells; proximal cells cylindrical or ellipsoidal; distal cells obovoidal or ellipsoidal; secondary fascicles present, but not covering the entire internode; carpogonial branches composed of 3-4 disc- or barrel shaped cells, arising from proximal cells of primary fascicles, short, 16-22 µm in length; carpogonia with sessile, elongate pear-shaped, elongate conical or irregularly shaped trichogynes, 25-40 µm in length, 7-11

µm in diameter; gonimoblast filaments with erect branches of one cell; carposporangia obovoidal, 9-10 µm in length, 6-7 µm in diameter.

Distribution: Africa – Zambia (known from the type locality only).

Representative DNA sequences: *rbc*L (MN974518).

Remarks: this species was recently described by Szinte *et al*. (2020) and it is most closely comparable to *S. huillensis* based on the reduced whorls (162-364 µm in diameter), shorter carposporangia (8-10 µm in length, respectively) and the occurrence in Africa. *Sirodotia kennedyi* differs from *S. huillensis* in number of primary fascicle cells, 3-5 versus 5-9(-10), respectively. In addition, they are genetically divergent, 2.1% for *rbc*L.

Sirodotia suecica Kylin, *Nova Acta Regiae Soc. Sci. Ups*., Ser IV, 3: 38 (1912). Figs. 48-52.

Homotypic synonym: *Batrachospermum suecicum* (Kylin) Necchi & Entwisle, *Phycologia* 29: 486 (1990).

Heterotypic synonyms: *Sirodotia fennica* Skuja, *Archiv für Protistenkunda* 74: 297 (1931); *S. sinica* C. C. Jao, *Sinensia* 12: 267-270 (1941); *Sirodotia tenuissima* (Collins) Skuja ex Flint, *American Journal of Botany* 35: 431 (1948); *Sirodotia acuminata* Skuja ex Flint, *American Journal of Botany* 37:755 (1951); *Sirodotia segawae* Kumano, *Botanical Magazine, Tokyo* 95: 128-131 (1982); *Sirodotia yutakae* Kumano, *Botanical Magazine, Tokyo* 95: 126-129 (1982); *Sirodotia goebelii* Entwisle & Foard, *Australian Systematic Botany* 12(4): 610 (1999).

Type: LD, H. Kylin, 3.viii.1909 (Lectotype); UPS A-653877(Isolectotype).

Type locality: Sweden, Skäne, Osby; 56º23’01”N, 13º59’34”E.

Revised description. Plants monoecious or dioecious; whorls (85-)135-850 µm in diameter; primary fascicles, 4-12(-13) cells; proximal cells cylindrical, obovoidal, ellipsoidal, fusiform or ovoidal; distal cells ellipsoidal, obovoidal or subspherical; secondary fascicles abundant, covering the entire internode; spermatangia spherical or ellipsoidal, 1-3, few or abundant on primary or secondary fascicles, (4-)5-9 µm in diameter; carpogonial branches composed of (1-)2-9 disc- or barrel shaped cells, short, 5-40 µm long, arising from periaxial, proximal or median cells of primary fascicles and cortical filaments, rarely on secondary fascicles; carpogonia with sessile, elongate cylindrical, ellipsoidal, elongate pear-shaped or irregularly shaped trichogynes, (15-)19-48 µm in length, (4-)5-16 µm in diameter; gonimoblast initial developing from the

non-protuberant side of the carpogonium; gonimoblast filaments with erect branches of 2-9 cells; carposporangia obovoidal, ellipsoidal, spherical or pear-shaped, 10-21 µm in length, 6-11 µm in diameter.

Additional specimen examined: BHO A-0264, SJRP 32581, SJRP 32582, SJRP 32583, NSW799516, MEL2033246A, WELT A027220 (Table S5).

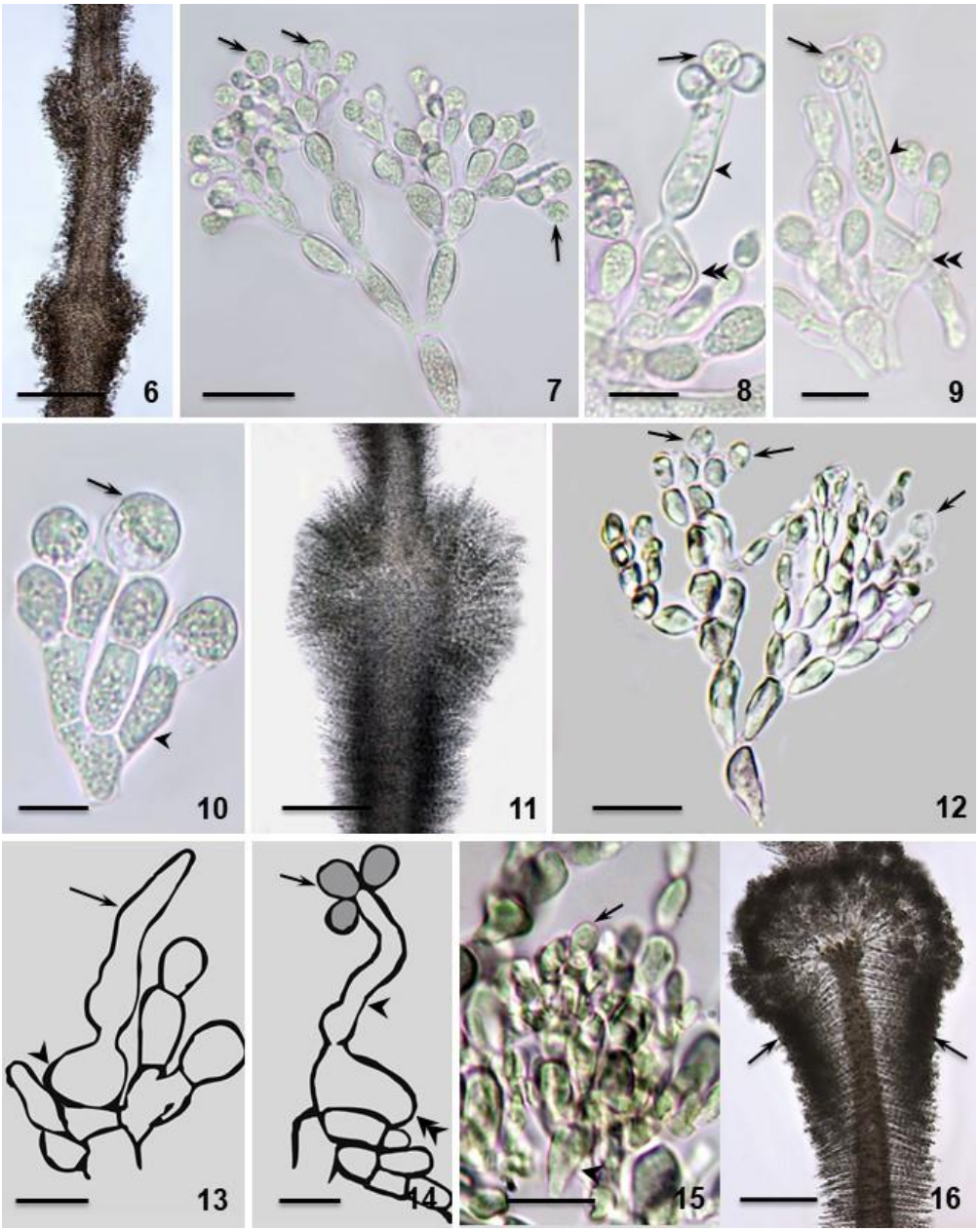
Distribution: Africa, Asia, Australasia, Europe and North America.

Representative DNA sequences: COI-5P (FI1**,** FI4, FI5) and *rbc*L (FI2, JF344724, JF344725).

Remarks: This species has a broad geographic distribution and a broad range for the morphological features. It is comparable to the other species with large carposporangia and spermatangia 1-3 on primary fascicles (e.g. *S. delicatula*, *S. delicatuliformis*, *S. amazonica*, *S. cryptica* and *S. aquiloamericana*). The species is clearly differentiated by the unique character of gonimoblast initial developing from the non-protuberant side of the carpogonium. Molecular data also showed the species to be distinct from all others in the genus (*rbc*L, COI-5P and LSU). The heterotypic synonyms considered in this study were based on the analysis of types, protologues and molecular data. The morphological characters used to distinguish these species (*S. fennica*, *S. sinica, S. tenuissima, S. acuminata*, S*. segawae*, *S. yutakae* and *S. goebelii*) overlap most characters (breeding system, internode length, carpogonial branch growth and length and carpogonia length) of *S. suecica* and they are not informative due to continuous variation in morphometrics. Therefore, they are considered to be synonymous based on the gonimoblast initial developing from the opposite side of the basal protuberance of carpogonium. In addition, molecular data for *S. tenuissima* and *S. goebelii* showed these taxa to have very similar *rbc*L sequences to *S. suecica* (LAM *et al*., 2012). Furthermore, based on the analysis of type specimens and protologues of *S. segawae* and *S. yutakae*, we did not confirm the existence of specialized spermatangial branches on primary fascicles and secondary fascicles (*S. yutakae*) or on specialized filaments on cortical filaments or on shortened involucral filaments of carpogonial branches (*S. segawae*). As well, the development of the initial gonimoblast filament from both sides of the basal protuberance of the carpogonium was not observed in any other species of the genus as reported in *S. segawae* and *S. sinica*, suggesting that this is a mistake, supposedly due to observation of mixed populations involving two or more species. Thus, we concluded that *S. segawae*, *S. yutakae* and *S. sinica* should be placed in synonymy with *S. suecica,* considering that the gonimoblast initial

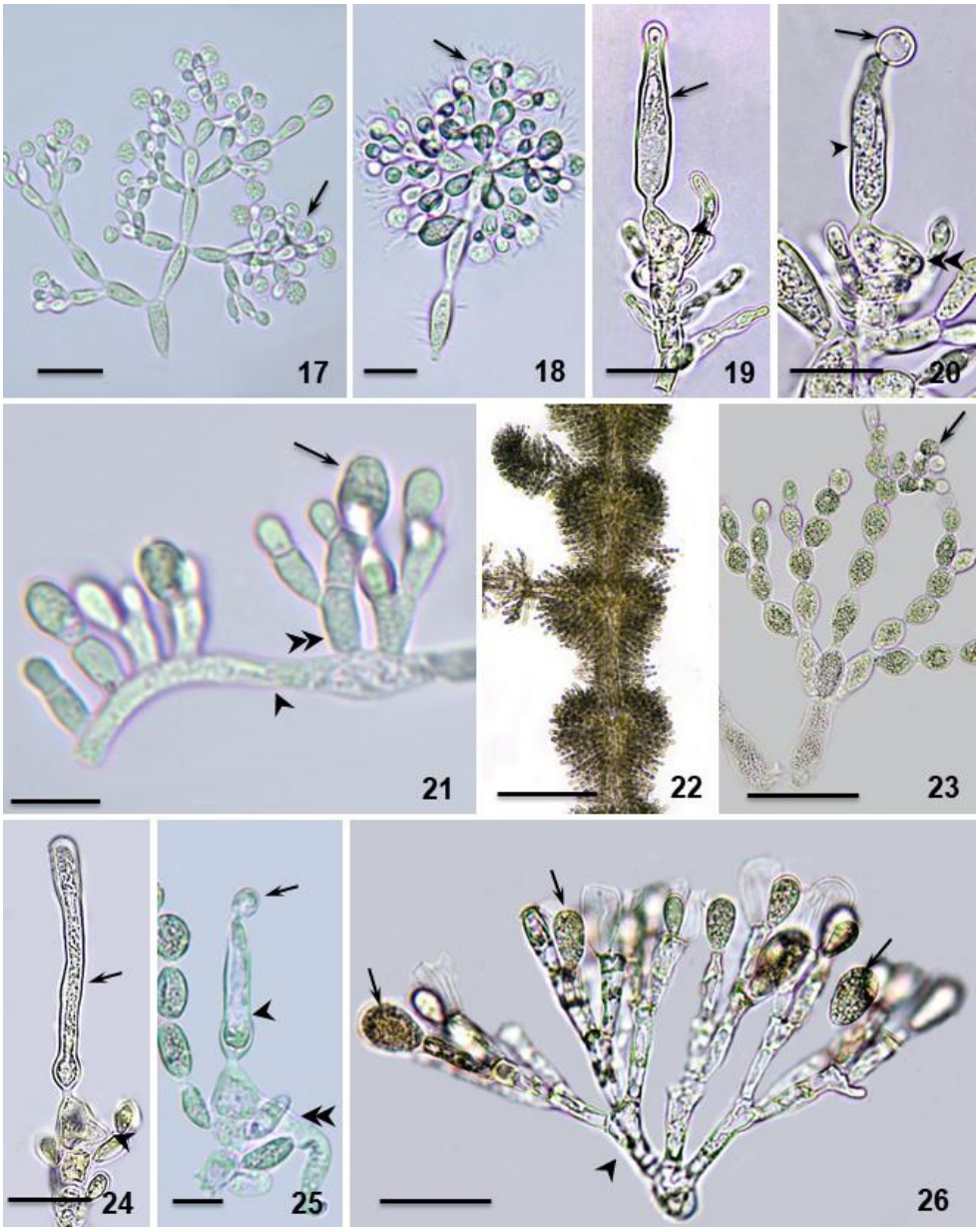
develops from the opposite side of the basal protuberance of carpogonium and it has a widespread geographic distribution.

Figs. 6-10. *Sirodotia amazonica* (photomicrographs of the holotype, SJRP 31924). **6.** Pear-shaped whorls. **7.** Primary fascicle with spermatangia (arrow). **8.** Carpogonium with a mature trichogyne (arrowhead) showing the basal hemispherical protuberance (double arrow) and attached spermatium (arrow). **9** Fertilized carpogonium with a cylindrical trichogyne (arrowhead), attached spermatium (arrow) and gonimoblast initial developing from the protuberant side of the carpogonial base (double arrow). **10**. Carposporangium (arrow) and erect (arrowhead) gonimoblast filament. Scale bars: 50 µm for figures 6; 20 µm for figure 7; 10 µm for figure 8-10. **Figs. 11-15**. *Sirodotia aquiloamericana* (photomicrographs of the holotype, BHO-0437). **11.** Pear-shaped whorls. **12**. Primary fascicle with spermatangium (arrow). **13.** Carpogonium with a mature trichogyne (arrow) showing the basal hemispherical protuberance (arrowhead). **14**. Fertilized carpogonium with a cylindrical trichogyne (arrowhead), attached spermatium (arrow) and gonimoblast initial developing from the protuberant side of the carpogonial base (double arrow). **15**. Carposporangium (arrow) and erect (arrowhead) gonimoblast filament. Scale bars: 200 µm for figures 11; 20 µm for figure 12 and 15 10 µm for figure 13-14. Figs. 13-14 redrawn from Necchi *et. al*. (1993).



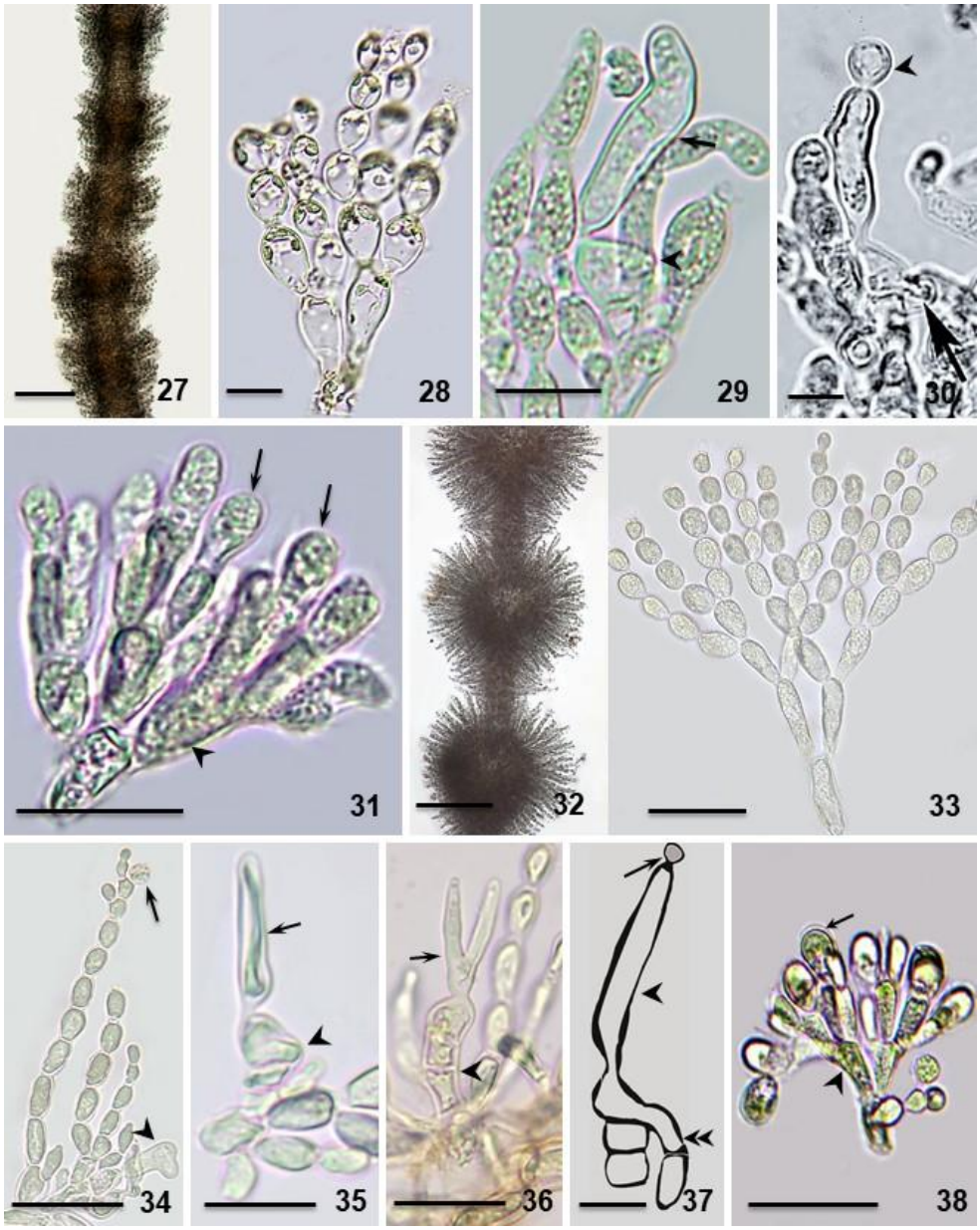
Source: Author's own production

Figs. 16-21. *Sirodotia assamica* (photomicrographs of SJRP 32583, SJRP 32584). **16**. Pear-shaped whorl showing densely arranged spermatangia (arrows). **17.** Primary fascicles and spermatangia (arrow) in clusters. **18.** Detail of spermatangia (arrow) in clusters on primary fascicle. **19.** A carpogonium with a mature trichogyne (arrow) showing the basal hemispherical protuberance (arrowhead). **20.** Fertilized carpogonium with attached spermatium (arrow) and gonimoblast initial (double arrowhead) developing from the same side of the basal protuberance (arrowhead). **21.** Carposporangia (arrow), prostrate (arrowhead) and erect (double arrowhead) gonimoblast filaments. Scale bars: 200 µm for figure 16; 20 µm for figures 17-20. 10 µm for figure 21. Figs 18-20 redrawn from Rossignolo *et al*. (2020). **Figs. 22-26**. *Sirodotia cryptica* (photomicrographs of the holotype, samples SJRP 32575). **22.** Obconic whorls. **23.** Primary fascicle with spermatangia (arrow). **24.** Carpogonium with a mature cylindrical trichogyne (arrow) showing the basal hemispherical protuberance (arrowhead). **25.** Carpogonium with a sessile elongate-conical trichogyne (arrowhead), attached spermatium (arrow) and gonimoblast initial developing from the protuberant side of the carpogonial base (double arrowhead). **26**. Carposporangia (arrow) and erect (arrowhead) gonimoblast filaments. Scale bars: 200 µm for figure 22; 50 µm for figure 23; 20 µm for figure 24 and 26; 10 µm for figure 25. Fig. 24 is from SJRP 31923.



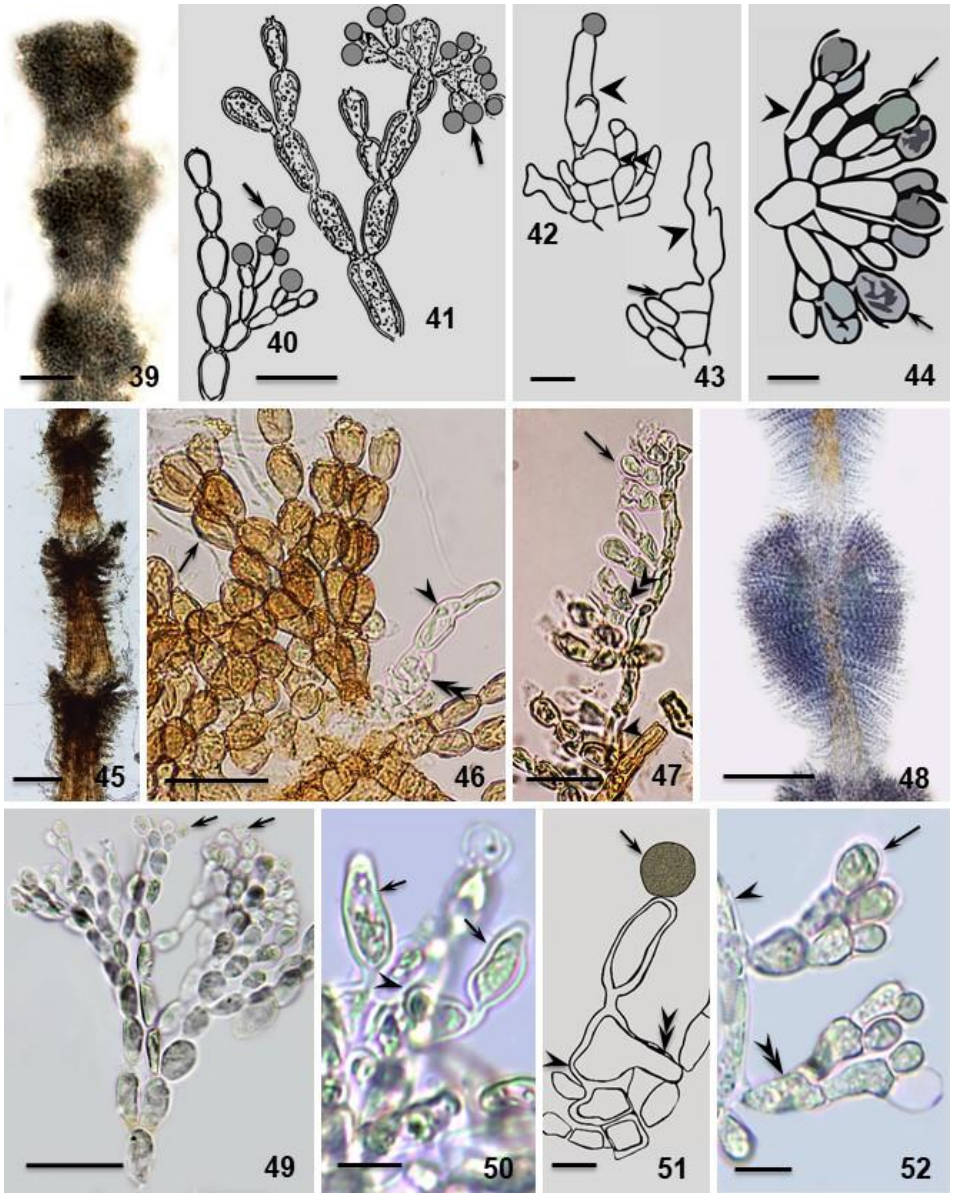
Source: Author's own production

Figs. 27-31*. Sirodotia delicatula (photomicrographs of BHO A-0984).* ***27.*** *Obconic whorls.* ***28.*** *Primary fascicle.* ***29.*** *Carpogonium with a mature trichogyne (arrow) showing the basal hemispherical protuberance (arrowhead).* ***30.*** *Carpogonium with a sessile cylindrical trichogyne, attached spermatium (arrowhead) and gonimoblast initial developing from the protuberant side of the carpogonial base (arrow).* ***31****. Carposporangia (arrow) and erect (arrowhead) gonimoblast filaments. Scale bars: 200 µm for figure 27; 20 µm for figures 28, 29 and 31. 10 µm for figure 30. Fig. 30 redrawn from Johnston et. al. (2014).* ***Figs. 32-38****. Sirodotia delicatuliformis (photomicrographs of holotype, SJRP 31918).* ***32.*** *Obconic whorls.* ***33.*** *Primary fascicle.* ***34.*** *Primary fascicle with espermatangia (arrow) and anomalous shape (bent end) trichogyne (arrowhead).* ***35.*** *Carpogonium with a mature trichogyne (arrow) showing the basal hemispherical protuberance (double arrow).* ***36.*** *Carpogonium with anomalous shape (bifurcate) trichogyne (arrow) and carpogonial branch (arrowhead).* ***37.*** *Carpogonium with a sessile cylindrical trichogyne (arrowhead), attached spermatium (arrow) and gonimoblast initial developing from the protuberant side of the carpogonial base (double arrowhead).* ***38****. Carposporangia (long arrow) and erect (short arrow) gonimoblast filaments. Scale bars: 200 µm for figure 32; 50 µm for figures 33, 34 and 36; 25 µm for figure 38; 20 µm for figure 35. 10 µm for figure 37. Fig 36 from SJRP 31919 (Table S5); Fig 37 redrawn from Necchi (1991).*



Source: Author's own production

Figs. 39-44. *Sirodotia huillensis* (photomicrographs of BHO A-1447). **39**. Obconic whorls. **40-41**. Primary fascicle with spermatangia (arrow). **42-43**. Carpogonium with irregular trichogyne (arrowhead) showing the basal hemispherical protuberance (double arrow) and gonimoblast initial developing from the protuberant side of the carpogonium (arrow). **44**. Carposporangia (arrow) and erect (arrowhead) gonimoblast filaments. Scale bars: 100 µm for figure 39; 20 µm for figures 40-41; 10 µm for figure 42-44. Figs 40-44 redrawn from Skuja (1931). **Figs. 45-47** *Sirodotia kennedyi* (photomicrographs of BHO A-0946). **45**. Obconic whorls. **46**. Primary fascicles (arrow) and carpogonium with irregular trichogyne (arowhead) showing the basal hemispherical protuberance (double arrow). **47**. Carposporangia (arrow), prostrate (arrowhead) and erect (double arrow) gonimoblast filaments. Scale bars: 100 µm for figure 45; 20 µm for figures 46-47. Figs. 45-47 redrawn from Szinte *et. al* (2020). **Figs. 48-52**. *Sirodotia suecica* Kylin (photomicrographs of BHO A-0266, SJRP32582, SJRP32583). **48.** Pear-shaped whorl. **49.** Primary fascicles and terminal spermatangia (arrow). **50.** Carpogonium with mature trichogyne (arrow) and a basal hemispherical protuberance (arrowhead). **51.** Fertilized carpogonium with attached spermatium (arrow) and gonimoblast initial (double arrow) on the opposite side of the basal protuberance (arrow). **52.** Carposporangia (arrow), prostrate (arrowhead) and erect (double arrow) gonimoblast filaments. Scale bars: 100 µm for figure 48; 50 µm for figure 49; 10 µm for figures 50-52. Fig. 51 redrawn from Necchi *et. al*. (1993).



Source: Author's own production

3.4.2.3. Doubtful species

The species listed below could not be confirmed if they represent a separate entity from any of the species recognized in this investigation. The reasons for the uncertainty were generally due the unavailability of the type specimen or descriptions that lack the primary diagnostic characters currently used for species identification. The name of each species is accompanied by the reasons that led us to treat it as a doubtful species. They generally represent species with a very limited geographic distribution, some known only by type localities.

Sirodotia cirrhosa Skuja ex MS Balakrishnan & BB Chaugule, *Indian Batrachospermaceae*: 242 (1980). It was listed as a validly published species (Guiry & Guiry 2020; Ganesan *et al*. 2018). However, the type specimen is not available for analysis.

Sirodotia gardneri Skuja ex L.Flint, *American Journal of Botany* 37: 754 (1951). Type specimen is not available for analysis. Possibly a distinct species within the group, however, the type specimen analyzed by Necchi *et al* (1993) was a male plant and therefore key characteristics could not be confirmed.

Sirodotia huangshanensis ZX Shi et SL Xie, *Journal of Tropical and Subtropical Botany* 12 (1): 2 (2004). Type specimen is not available for analysis; in the protologue the species was distinguished based on breeding system (monoecious) and the presence of monosporangia in the secondary fascicles. In *Sirodotia*, it has been shown that the distinction of species based on breeding system (monoecious, dioecious or polyoecious) is not a reliable taxonomic character (Umezaki, 1960). The presence of monosporangia may be a misinterpretation of carposporangia based on size and position shown in the protologue.

3.5. DISCUSSION

Concatenated phylogenetic and single gene analyses based on a representative number of populations of each species and geographic regions showed that the genus *Sirodotia* represents a monophyletic group. These results corroborated general studies of the order (VIS *et al*., 1998; VIS & SHEATH, 1999; ENTWISLE *et al*., 2009) and specific investigations for the genus (NECCHI *et al*., 2007; LAM *et al*., 2012; PAIANO & NECCHI, 2013). In terms of diversity, 23 species names were listed in the

genus before this study (OTT, 2009; GUIRY & GUIRY, 2020), of which 13 were currently accepted taxonomically and three species were reported as widespread, *S. suecica*, *S. huillensis* and *S. delicatula* (SKUJA, 1931, 1938b; JAO, 1941; ISRAELSON, 1942; FLINT, 1948, 1950; REIS, 1969; UMEZAKI, 1960; SHEATH & HYMES, 1980; ENTWISLE & KRAFT, 1982; NECCHI, 1990; NECCHI *et* al., 1993; KUMANO, 2002; LAM *et al*., 2012; PAIANO *et al*., 2013). Three species that were accepted taxonomically (*S. cirrhosa*, *S. gardneri* and *S. huangshanensis*) could not be confirmed as separate entities because the type specimens were not available for analysis or the characters used to describe them were not of taxonomic value. Four species (*S. iyengarii, S. polygama, S. angolensis* and *S. nigrescens*) were removed from the genus *Sirodotia* for having characteristics of other genera (*Kumanoa*, *Paludicola* and *Torularia*; WYNNE, 2019; VIS *et al.,* 2020). Three species (*S. segawae*, *S. sinica* and *S. yutakae*) are here proposed as synonyms with *S. suecica,* whereas *S. ateleia* is synonymized with *S. delicatula.* Four species (*S. acuminata*, *S. fennica*, *S. goebelii* and *S. tenuissima*) have already been synonymized with *S. suecica*. There are three species (*S. ambigua*, *S. loefgrenii* and *S. moniliformis*) that are known manuscript names by H. Skuja but have not been published and therefore are not taxonomically valid (OTT, 2009). We recognized three species that were already described (*S. delicatula*, *S. huillensis*, *S. suecica*) and two recently described (*S. assamica* and *S. kennedyi*) based on molecular data, morphological characters and geographical distribution. Four additional *Sirodotia* species were here proposed as new: *S. amazonica*, *S. aquiloamericana*, *S. cryptica* and *S. delicatuliformis*. Some of the species are reported from a single continent or restricted regions and thus biogeography can be used along with morphology as an additional criterion, if DNA sequence data are not available. The following species exhibited a well-defined geographical pattern: *S. delicatuliformis* from Brazil and Costa Rica, *S. amazonica* (midwestern and norther region of Brazil) and *S. cryptica* (midwestern of Brazil), *S. assamica* from northeastern India, *S. aquiloamericana* from southern North America, *S. huillensis* from southern and southeastern Africa, *S. kennedyi* from south-central Africa.

The levels of intraspecific divergence for *rbc*L and COI-5P sequences observed in sequences of *Sirodotia* (0-2.4% and 0-3.8%, respectively) are within the variation within species previously observed in other genera of the order Batrachospermales (AGOSTINHO *et al*., 2014; ROSSIGNOLO & NECCHI, 2016; NECCHI *et al*., 2018,

2019; VIS *et al*., 2020). The interspecific divergence values for *rbc*L and COI-5P were relatively high (1.6-8.1% for *rbc*L and 4.2-10.5% for COI-5P). The three markers (*rbc*L, COI-5P and LSU) used in this study revealed relatively similar results in that the trees resulting from the concatenated analysis and of single genes showed nine distinct species some with clear biogeographic partition as well as cryptic species. Concatenated and single gene analysis (*rbc*L and COI-5P) showed trees with high support and relatively clear cut inter and intraspecific divergence values. In contrast, the LSU analysis showed lower divergence and support values and therefore, its applicability as a species barcode is not as effective as the COI-5P sequences. The LSU marker is very conserved in relation to the other two used in this investigation and it is not widely used for species identification. Full LSU sequences have been successfully used to infer phylogenies in single gene analyses or combined with other markers (*rbc*L, UPA, SSU) at the family and order levels (HARPER & SAUNDERS, 2002; HUISMAN *et al*., 2004; TRONCHIN *et al*., 2004; SHERWOOD *et al*., 2010). Thus, the use of the LSU marker in this study served more precisely to compose the concatenated analysis of three markers and provided greater support for the clades in the phylogenetic tree.

All samples of *Sirodotia* from Brazil were previously treated as a single species, *S. delicatula* (NECCHI, 1991; NECCHI *et al*., 1993, 2007; VIS & SHEATH, 1999; LAM *et al*., 2012). However, a recent study (PAIANO & NECCHI, 2013) confirmed the existence of cryptic species of *Sirodotia* in Brazil and that there are at least three genetically distinct species. Jonhston *et al*. (2014) reported *rbc*L sequence of *S. delicatula* from Malaysia (near the type locality - Indonesia, SKUJA 1938b), that is very divergent (4.4-5.0%) from the Brazilian populations identified as *S. delicatula*. The sequence from Malaysia is considered represents *S. delicatula*. Therefore, our results confirmed that Brazilian specimens are distinct from *S. delicatula* and are here described as three new species: *S. amazonica*, *S. cryptica* and *S. delicatuliformis*.

The diagnostic morphological characters used for distinghishing the genus (asymmetric carpogonial base with protuberance and diffuse carposporophyte with prostrate filaments producing determinate erect branches with carposporangia) in previous studies (NECCHI, 1991; NECCHI *et al*., 1993; VIS & SHEATH, 1999; KUMANO, 2002; NECCHI *et al*., 2007; LAM *et al*., 2012) were confirmed as good criteria. On the other hand, some morphological characters previously utilized to distinguish species in the genus *Sirodotia* were here considered as possibly

misinterpreted or taxonomically uninformative. Kumano (1982) used the presence of spermatangia on specialized branches to distinguish *S. yutakae* and *S. segawae*. However, based on the figure in the protologue and our examination of type specimens, we interpreted them as smaller distal cells of fascicles or smaller cells at the tips of the involucral filaments. A second character reported in species of the genus is the origin of carpogonial branches arising from periaxial, intercalary and terminal cells of primary fascicles and cortical filaments observed in *S. sinica* (JAO, 1941). However, in this study such distinct origins of the carpogonial branches were observed in almost all species recognized in the genus. A third character used as diagnostic within the genus is the length of carpogonia. Kumano (2002), Necchi *et al*. (2007) and Lam *et al*. (2012) used the length of the carpogonium to distinguish *S. suecica* (30-40 µm long), *S. gardneri* (20 µm long), *S. huillensis* (37-53 µm long) and *S. delicatula* (25-35 µm long) from other species based on observations in the type specimens and samples collected in other regions. However, the length of the carpogonium has a much wider variation and greatly overlaps among species of the genus (Tables S8-S10). Other character applied (carpogonium curved or deflexed, *S. goebelii*) by Kumano (2002) to distinguish species in the genus have no taxonomic value as it occurs in several species. The presence of monosporangia at the tips of secondary fascicles was observed in *S. huangshanensis* (XIE & SHI, 2004), who argued it was a diagnostic character. Based on the figures in the protologue, we noted that size and position of monosporangia resemble carposporangia and we suggested that carposporangia might have been misinterpreted as monosporangia. In all species of *Sirodotia*, it has been shown that the distinction of species based on breeding system (monoecious or dioecious) is not a reliable character, because mixed populations of monoecious and dioecious thalli have been reported in *Sirodotia* species (NECCHI, 1991). In addition, as pointed out by Umezaki (1960), the separation of sex should not be considered important in the specific distinction within the family Batrachospermaceae because polygamy is very common.

In summary, the following morphological characters were applied to distinguish species in combination with DNA sequence data and geographic distribution: 1) primary fascicle cell number short (3-5, *S. kennedyi*) or long (remaining species); 2) spermatangia arranged in clusters on the fascicles (*S. assamica*); 3) origin of gonimoblast filament in the carpogonium (*S. suecica*, on the opposite side of the basal protuberance or from the protuberance in all other species); 4) size of carposporangia

– narrow (6-8.5(-9.5) µm in diameter) vs wide (8-13 µm in diameter) and long (10-21 µm in length) vs short (8-10 µm in length).

In this study, we evaluated previously assigned and newly collected specimens of the genus *Sirodotia* using DNA sequence and morphological data from a wide geographical range. The results highlighted a pattern of high genetic diversity in *Sirodotia* with new information on the species richness and the biogeography of the genus. In summary, this research synthesized and organized the information, clarifying the taxonomy and providing biogeographic information for the species.

Acknowledgements

Rossignolo and Necchi are grateful to the Brazilian agencies FAPESP (2016/07808-1, 2016/16320-205 2) and CNPq (302415/2017-3) for financial support by grants and scholarship. We are grateful to the herbarium curators (LISU, TNS-AL and UPS) to allow access to the type specimens.

3.6. REFERENCES

ABDELAHAD, N.; BOLPAGNI, R.; LASINIO, G.J.L.; VIS, M.L.; AMADIO, C.; LAINI, A.; KEIL, J.E. Distribution, morphology and ecological niche of *Batrachospermum* and *Sheathia* species (Batrachospermales, Rhodophyta) in the *fontanili* of the Po plain (northern Italy). **European Journal of Phycology,** v. 50, p. 318-329, 2015.

AGOSTINHO, D.C.; NECCHI, O. JR. Systematics of the section *Virescentia* of the genus *Batrachospermum* (Batrachospermales, Rhodophyta) in Brazil. **Phycologia***,* v. 53, p. 561–570, 2014.

BALAKRISHNAN, M.S.; CHAUGULE, B.B. Indian Batrachospermaceae. *In*: **Papers presented at the International Symposium on Taxonomy of Algae held at the Centre of Advanced Study in Botany University of Madras**, December 9-16, 1974. Edited by: T.V. Desikachary, and V.N. Raja Rao. University of Madras, Madras, 1980. pp. 223-248.

BALUSWAMI, M.; BABU, M. The structure and reproduction of *Sirodotia iyengarii sp. nov. In:* Subba-Rangiah, G. (Ed.) **Recent Trends in Algal Research**. Department of Botany, Andhra University, Waltair, 1999, p. 237–244.

BENSON, D. A.; CAVANAUGH, M.; CLARK, K.; OSTELL, J.; WHEELER, D.L. GenBank. **Nucleic acids Research**, v. 41, p. 36-42, 2013.

CARMONA, J.J.; MONTEJANO, G.Z.; NECCHI, O. JR. The ecology and morphological characterization of gametophyte and “Chantransia” stage of *Sirodotia*

huillensis (Batrachospermales, Rhodophyta) from a stream in Central Mexico. **Phycological Research***,* v. 54, p. 108–115, 2006.

CHAPUIS, I.S.; NECCHI, O. JR.; ZUCARRELLO, G.C.; XIE, S.L.; ABOAL, M.; SÁNCHEZ CASTILHO, P. M.; VIS, M.L. A new genus, *Volatus* and four new species of *Batrachospermum* sensu strict (Batrachospermales, Rhodophyta). **Phycologia,** v. 56, p. 454-468, 2017.

DARRIBA, D.; TABOADA, G.L.; DOALLO, R.; POSADA, D. jModelTest 2: more models, new heuristics and parallel computing. **Nature Methods**, *v.* 9, p. 772, 2012.

ELORANTA, P.; KWANDRANS, J. Freshwater red algae (Rhodophyta): identification guide and to European taxa, particularly to those in Finland**. Norrlinia,** v. 15, p. 1–103, 2007.

ELORANTA P., KWANDRANS, J.; KUSEL-FETZMANN, E. *Rhodophyta and Phaeophyceae*. *In* **Freshwater Flora of Central Europe**, Heidelberg: Spectrum Akademischer Verlag, 2011. p. 57–59.

ENTWISLE, T.J.; FOARD, H.J. *Sirodotia* (Batrachospermales, Rhodophyta) in Australia and New Zealand. **Australian Systematic Botany**, v. 10, p. 605–613, 1999.

ENTWISLE, T.J.; KRAFT, G.T. Survey of freshwater red algae (Rhodophyta) of southeastern Australia. **Australian Journal of Marine and Freshwater Research**, v. 35, p. 213 – 259, 1982.

ENTWISLE, T.J.; JOHNSTON, E.T.; LAM, D.W.; STEWART, S.A.; VIS, M.L. *Nocturama* gen. nov., *Nothocladus* s. lat. and other taxonomic novelties resulting from the further resolution of paraphyly in Australasian members of *Batrachospermum* (Batrachospermales, Rhodophyta). **Journal of Phycology***,* v. 52, p. 384-396, 2016.

ENTWISLE, T.J.; VIS, M.L.; CHIASSON, W.B.; NECCHI, O. JR.; SHERWOOD A.R. Systematics of the Batrachospermales-A Synthesis. **Journal of Phycology**, v*.* 45, p. 704–715, 2009.

EVANS, J.R.; CHAPUIS, I.S.; VIS, M.L. Adding to the freshwater red algal diversitu in North America: *Lympha mucosa* gen. et sp. nov. (Batrachospermlaes, Rhodophyta). **Algae**, v. 32, p. 171-179, 2017.

FLINT, L.H. Studies on the freshwater red algae. **American Journal of Botany**, v. 35, p. 428-433, 1948.

FLINT, L.H. Studies on the freshwater red algae. **American Journal of Botany**, v. 37, p. 754-757, 1950. 1951.

GANESAN, E.K.; WEST, J.A.; NECCHI, O. JR. A catalogue and bibliography of non-marine (freshwater and estuarine) Rhodophyta (red algae) of India. **Phytotaxa**, v.1, p.1–48, 2018.

GUIRY, M.D.; GUIRY, G.M. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed 18 July 2020.

HARPER, J.T.; SAUNDERS, G.W. The application of sequences of the ribosomal cistron to the systematics and classification of the florideophyte red algae (Florideophyceae, Rhodophyta). **Cahiers de Biologie Marine***,* v. 42, p. 25–28, 2001.

HUISMAN, J.M.; ABBOTT, L.A.; SHERWOOD, A.R. Large subunit rDNA gene sequences and reproductive morphology reveal Stenopeltis to be a member of the Liagoraceae (Nemaliales, Rhodophyta), with a description of Akalaphycus gen. nov. **European Journal of Phycology**, v. 39, p. 257-272, 2004.

ISRAELSON, G. The freshwater Florideae of Sweden. Studies on their taxonomy, ecology and distribution. **Symbolae Botanicae Upsaliensis**, v. 6, p. 1–134, 1942.

JAO,C.C. Studies on the freshwater algae of ChinaVIII. A preliminary account of the Chinese freshwater Rhodophyceae. **Sinensia**, v. 15, p. 61-92, 1941.

JOHNSTON, E.T.; LIM, P.-E.; BUHARI, N.; KEIL, E.J.; DJAWAD, M.I.; VIS, M.L. Diversity of freshwater red algae (Rhodophyta) in Malaysia and Indonesia from morphological and molecular data. **Phycologia***,* v. 53, p. 329–341, 2014.

KACZMARCZYK, D.; SHEATH, R.G.; COLE, K.L. Distribution and systematics of the freshwater genus *Tuomeya* (Rhodophyta, Batrachospermaceae). **Jornal of Phycology,** v. 28, p. 850-855, 1992.

KEARSE, M.; MOIR, R.; WILSON, A.; STONES-HAVAS, S.; CHEUNG, M.; STURROCK, S.; BUXTON, S.; COOPER, A.; MARKOWITZ, S.; DURAN, C.; THIERER, T.; ASHTON, B.; MENTJIES, P.; DRUMMOND, A. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. **Bioinformatics**, v. 8, p. 1647-1649, 2012.

KUMANO, S. Four taxa of sections *Moniliformia, Hybrida* and *Setacea* of the genus *Batrachospermum* from temperate Japan. **Japanese Journal of Phycology**, v. 30, p. 289–296, 1982.

KUMANO, S. **Freshwater red algae of the world***.* Biopress Ltd., Bristol. 2002. p. 1-375.

KUMAR, S.; STECHER, G.; LI, M.; KNYAZ, C.; TAMURA, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. **Molecular Biology and Evolution***,* v. 35, p. 1547–1549, 2018.

KYLIN, H. Studien über die Schwedischen Arten der Gattungen *Batrachospermum* und *Sirodotia* nov. gen. **Nova Acata Regiae Societatis Scentiarum Upsaliensis**, v. 4, nº 3, p. 1–40, 1912.

LAM, D.W.; ENTWISLE, T.J.; ELORANTA, P.; KWANDRAS, J.; VIS, M.L. Circumscription of species in the genus *Sirodotia* (Batrachospermales, Rhodophyta) based on molecular and morphological data. **European Journal of Phycology**, v. 47, p. 42–50, 2012.

LELIAERT, F.; VERBRUGGEN, H.; VANORMELINGEN, P.; STEEN, F.; LÓPEZ BAUTISTA, J.M.; ZUCCARELLO, G.C.; DE CLERCK, O. DNA based species delimitation in algae. **European Journal of Phycology**, v. 49, p. 179–196, 2014.

PERUMAL, U.E.; RANI, V.U.; CHAUGULE, B.B. Studies on a red alga *Sirodotia huillensis* (Batrachospermaceae) from Orissa State, India. **Journal of Theoretical and Experimental Biology**, v. 11, p. 45–50, 2015.

NECCHI, O. Jr. Rhodophyta de água doce do estado de São Paulo: levantamento taxonômico. **Boletim de Botânica***,* v. 11, p. 11-69, 1989.

NECCHI, O. Jr. Revision of the genus *Batrachospermum* Roth (Rhodophyta, Batrachospermales) in Brazil. **Berlin: J. Cramer**, 1990. p. 201 p. (Bibliotheca Phycologica, 84).

NECCHI, O. JR. The Section *Sirodotia* of *Batrachospermum* (Rhodophyta, Batrachospermales) in Brazil. **Archiv fur Hydrobiologie**. Stuttgart: E Schweizerbart'sche Verlags, p. 17-30, 1991.

NECCHI, O. JR.; SHEATH, R.G.; COLE, K.M. Distribution and systematics of the genus *Sirodotia* (Batrachospermales, Rhodophyta) in North America. **Journal of Phycology**, v. 29, p. 236–243, 1993.

NECCHI, O. JR.; VIS, M.L.; OLIVEIRA, M.C. Phylogenetic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North and South America. **Cryptogamie, Algologie***,* v. 27, p. 117–127, 2007.

NECCHI, O. Jr.; VIS, M.L. **Monograph of the genus *Kumanoa* (Rhodophyta, Batrachospermales**. Stuttgart, J. Cramer, 2012. 79 p (Bibliotheca Phycologica 116)

NECCHI, O. JR.; AGOSTINHO, D.C.; VIS, M.L. Revision of *Batrachospermum* Section *Virescentia* (Batrachospermales, Rhodophyta) with the Establishment of the new genus, *Virescentia* stat. nov. **Cryptogamie, Algologie***,* v. 39, p. 313–338, 2018.

NECCHI, O. JR.; GARCIA FO., A. S.; PAIANO, M.O. Revision of *Batrachospermum* sections *Acarposporophytum* and *Aristata* (Batrachospermales, Rhodophyta) with the establishment of the new genera *Acarposporophycos* and *Visia.* **Phytotaxa**, v. 395, p. 051-065, 2019a.

NECCHI, O. JR.; GARCIA FO., A. S.; PAIANO, M.O.; VIS, M.L. Revision of *Batrachospermum* section *Macrospora* (Batrachospermales, Rhodophyta) with the establishment of the new genus *Montagnia*. **Phycologia***,* v. 58, p. 582–591, 2019b.

OTT, F.D. **Handbook of the taxonomic names associated with the non-marine Rhodophycophyta**. J. Cramer, Stuttgart, 2009. 969 pp.

PAIANO, M.O.; NECCHI, O. JR. Phylogeography of the freshwater red alga *Sirodotia* (Batrachospermales, Rhodophyta) in Brazil. **Phycological Research**, v. 61, p. 249–255, 2013.

PUILLANDRE N., LAMBERT A., BROUILLET S. & ACHAZ G. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. **Molecular Ecology**, v. 21, p. 1864–1877, 2012.

REIS, M.P. Subsídios para o conhecimento das rodofíceas de água doce de Portugal V. **Boletim da Sociedade Broteriana**, v. 43, p. 183-192, 1969.

RAMBAUT, A.; DRUMMOND, A.J.; XIE, D.; BAELE, G.; SUCHARD, M.A. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. **Systematic Biology**, v. 67, p. 901–904, 2018.

RONQUIST, F.; TESLENKO, M.; VAN DER MARK, P.; AYRES, D.L.; DARLING, A.; HÖHNA, S.; LARGET, B.; LIU, L.; SUCHARD, M.A.; HUELSENBECK, J.P. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. **Systematic Biology***,* v. 61, p. 539–542, 2012.

ROSSIGNOLO, N.L.; NECCHI, O. JR. Revision of section *Setacea* of the genus *Batrachospermum* (Batrachospermales, Rhodophyta) with emphasis on specimens from Brazil. **Phycologia***,* v. 55, p. 337–346, 2016.

ROSSIGNOLO, N.L.; YASMIN, F.; WEST, J.A.; GANESAN, E.K.; NECCHI, O. JR. Molecular and morphological evidence for a new species in the genus *Sirodotia* (Batrachospermales, Rhodophyta) from the State of Assam, India. **Phytotaxa**, v. 437, p. 121-134, 2020.

SALOMAKI, E.D.; KWANDRANS, J.; ELORANTA, P.; VIS, M.L. Molecular and morphological evidence for *Sheathia* gen. nov. (Batrachospermales, Rhodophyta) and three new species. **Journal of Phycology***,* v. 50, p. 526–42, 2014.

SAUNDERS, G.W. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future application. **Philosophical Transactions of the Royal Society**, v. 360, p. 1879–1888, 2005.

SAUNDERS, G.W.; MOORE, T.E. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. **Algae***,* v. 28, p. 31-43, 2013.

SAUNDERS, G.W.; NECCHI, O. JR. Nuclear rDNa sequences from Ballia prieurii support recognition of *Balliopsis* gen. nov. in the Batrachospermales (Florideophyceae, Rhodophyta). **Phycologia***,* v. 41, p. 61-67, 2002.

SHEATH, R.G. The biology of freshwater red algae. *In* **Progress in Phycological Research***.* Biopress, Bristol, v. 3, p. 89-157, 1984.

SHEATH, R.G.; HYMES, B.J. A preliminary investigation of the freshwater red algae in streams of southern Ontario, Canada. **Canadian Journal of Botany**, v. 58, p. 1295-1318, 1980.

SHEATH, R.G.; MÜLLER, K.M.; WHITTICK, A.; ENTWISLE, T.J. A re-examination of the morphology and reproduction of *Nothocladus lindaueri* (Batrachospermales, Rhodophyta). **Phycological Research***,* v. 44, p. 1-10, 1996.

SHEATH, R.G.; SHERWOOD, A.R. Phylum Rhodophyta (Red Algae). *In*: John D.M.; Whitton B.A. & Brook, A.J. (eds): **The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae**. Cambridge University Press, Cambridge. 2002. p. 124–143.

SHERWOOD, A.R.; SAUVAGE, T.; KURIHARA, A.; CONKLIN, K.Y.; PRESTING G.G. A comparative analysis of COI, LSU and UPA marker data for the Hawaiian florideophyte Rhodophyta: implications for DNA barcoding of red algae. **Cryptogamie Algologie***,* v. 31, p. 451-465, 2010.

SILVESTRO, D.; MICHALAK, I. RAxML GUI: a graphical front-end for RAxML. **Organism Diversity and Evolution***,* v.12, p. 335–337, 2012.

SKUJA, H. Untersuchungen über die Rhodophyceen des Süsswassers, 1-2. **Archiv für Protistenkunde,** v. 74, p. 297–309, 1931.

SKUJA, H. Comments on freshwater Rhodophyceae. **Bot. Rev.,** v. 4, p. 665-676, 1938a.

SKUJA, H. Die Susswasser rhodophyceen der Deutschen Limnologischen Sunda-Expedition. **Archiv für Hydrobiologie***,* v.15, p. 603-637, 1938b.

SOUTHWOOD, K. E. Substantive theory and statistical interactions: Five models. **American Journal of Sociology**, v. 83, p. 1154–1203, 1978.

STAMATAKIS, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. **Bioinformatics***,* v. 30, p. 1312–3, 2014.

SZINTE A.L., TAYLOR J.C., ABOSEDE A.T. & VIS M.L. Current status of freshwater red algal diversity (Rhodophyta) of the African continent including description of new taxa (Batrachospermales). **Phycologia**, v. 59, p. 1-13, 2020.

THIERS, B. **Index herbariorum: a global directory of public herbaria and associated staff.** New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/2020 (continuously updated).

TRONCHIN, E. M.; DE CLERCK, O.; FRESHWATER, D.W.; BOLTON, J.J.; ANDERSON, R.J. *Ptilophora leliaertii* and *Ptilophora coppejansii*, two new species of Gelidiales (Rhodophyta) from South Africa. **European Journal of Phycology**, v. 39, p. 395-410, 2004.

UMEZAKI, I. On *Sirodotia delicatula* Skuja from Japan. **Acta Phytotaxonomica et Geobotanica**, v. 18, p. 208-214, 1960.

VIS, M.L.; HARPER, J.T.; SAUNDERS, G.W. Large subunit rDNA and *rbc*L gene sequence data place *Petrohua bernabei* gen. et sp. nov. in the Batrachospermales (Rhodophyta), but do not provide further resolution among taxa in this order. **Phycological Ressearch***,* v. 55, p. 103-112, 2007.

VIS, M.L.; SAUNDERS, G.W.; SHEATH, R.G.; DUNSE, K.; ENTWISLE T.J. Phylogeny of the Batrachospermales (Rhodophyta) as inferred from *rbc*L and 18S ribosomal RNA gene DNA sequences. **Journal of Phycology***,* v. 34, p. 341-350, 1998.

VIS, M.L.; SHEATH, R.G. A molecular investigation of the systematic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North America. **Phycologia***,* v. 38, p. 261–266, 1999.

VIS, M.L.; LEE, J.; ELORANTA, P.; CHAPUIS, I.S.; LAM, D.W.; NECCHI, O. JR. *Paludicola* gen. nov. and revision of the species formerly in *Batrachospermum* section *Turfosa* (Batrachospermales, Rhodophyta). **Jornal of Phycology***,* 2020.

WYNNE, M.J. *Torularia* Bonnnemaison, 1828, a generic name to be reinstated for *Atrophycus* Necchi & Rossignolo, 2017. **Notulae Algarum**, v. 89, p. 1-4, 2019.

XIE, S.L.; SHI, Z.X. Taxonomy of algal genus *Sirodotia* Kylin (Batrachospermaceae, Rhodophyta) in China. **Journal of Tropical and Subtropical Botany**, v. 12, p. 1-6, 2004.

3.7. SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S5. Species, collection information, and GenBank accession numbers for specimens of *Sirodotia* used in this study. New sequences in bold. Superscript letters denote identical sequences.

Table S6. Sequences of *rbc*L, COI-5P and LSU *Sirodotia* species obtained from Genbank used in the analyzes.

Table S7. Outgroup sequences used in *rbc*L, COI and LSU concatenated alignment for phylogenetic analyses.

Table S8. Morphological characters of *Sirodotia* specimens examined in this study or reported in relevant works. Sample code according to Table S5. For numerical data, the values (µm) represent the amplitude of variation (upper line) and mean ± standard deviation (lower line).

Table S9. Reproductive characters of *Sirodotia* specimens examined in this study or reported in relevant works. Sample code as Table S5. For numerical data the values (µm) represent amplitude of variation (upper line) and mean ± standard deviation (lower line).

Table S10. Comparative morphological characters and geographic distribution of some species of *Sirodoti*a. The dimension data are presented in µm.

Fig. S1. Maximum likelihood phylogenetic tree based on *rbc*L. The numbers associated with the nodes indicate the bootstrap values (BS) for maximum likelihood and posterior probability (PP) for Bayesian analysis; nodes without values indicate BS ≤ 70% and PP≤ 0.70. Sample codes as in Table S5.

Fig. S2. Distance tree (neighbor-joining) based on COI-5P sequences. * = bootstrap values > 95; nodes without values indicate bootstrap values ≤ 70. Information on the sequences are listed in Table S9-S10 (Supporting Information). Scale represents substitutions per site.

Fig. S3. Graphical representation of the results of Automatic Barcode Gap Discovery (ABGD) for the COI-5P sequences of the *Sirodotia* genus used in this study. A.

Histogram of the distribution of the different distance values for each species. B. The same distance data represented as ordered values.

Fig. S4. Distance tree (neighbor-joining) based on LSU sequences. * = bootstrap values > 95; nodes without values indicate bootstrap values ≤ 70. Information on the sequences are listed in Table S5-S6 (Supporting Information). Scale represents substitutions per site.

Table S5. Species, collection information, and GenBank accession numbers for specimens of *Sirodotia* used in this study. New sequences in bold. Superscript letters denote identical sequences.

Species/Code	Locality, collector, collection date, and herbarium voucher.	GenBank number		
		COI-5P	*rbc*L	LSU
South America/ Brazil				
S. delicatuliformis – BR1	Brazil: São Paulo, Paraguaçu Paulista, Maracaí, Córrego Km 487-488 (SP-374), 22°33'08"S, 50°48'26"W; O. Necchi Jr., 16.xi.2007 (SJRP 31915).	KF010483	KC951855[A]	–
S. delicatuliformis – BR2	Brazil: São Paulo, Paraibuna, Pousada Alto da Serra, 23°33'48''S, 45°27'31''W; O. Necchi Jr., 23.ix.2006 (SJRP 31916).	**This study**	KC951856[A]	**This study**
S. delicatuliformis – BR3	Brazil: São Paulo, Guapiaçu, close to the city, Córrego Cavenague, 20°47'14"S, 49°13'23"W; O. Necchi Jr., 02.vi.2008 (SJRP 31917).	KF010483	KC951857[A]	**This study**
S. delicatuliformis – BR4	Brazil: São Paulo, Mirassol, Rio S.J. dos Dourados, next to the fishing Aporé, 20°48'45"S, 49°34'29"W; O. Necchi Jr. 25.vi.2008 (SJRP 31918).	KF010486	KC951858[A]	**This study**
S. delicatuliformis – BR5	Brazil: São Paulo, Américo de Campos, Ribeirão Águas Paradas, 20°17'41"S, 49°46'35"W; O. Necchi Jr. 01.vi.2009 (SJRP 31919).	KF010483	KC951859[A]	**This study**
S. delicatuliformis – BR6	Brazil: São Paulo, São José do Rio Preto, stream Lagoa, 49°20'00''W; 20°49'00''S, 49°20'00"W; O. Necchi Jr. & Branco, L.H.Z, 27.viii.1999 (SJRP 23450).	–	KC951860[A]	–
S. delicatuliformis – BR7	Brazil: São Paulo, São José do Rio Preto, stream Talhadinho, 20°43'00''S, 49°13'00''W; O. Necchi Jr. & Branco, L.H.Z., 05.ix.1999 (SJRP 23501).	**This study**	KC951861[A]	**This study**
S. delicatuliformis – BR8	Brazil: Espirito Santo, Domingos Martins, Pedra Azul, Estrada Rota do Lagarto, 20°24'49"S, 41°02'23"W; O. Necchi Jr., 05.vii.2009 (SJRP 31920).	KF010487	KC951862[B]	**This study**
S. delicatuliformis – BR9	Brazil: Espirito Santo, Domingos Martins, Pedra Azul, Rio Jacu (Braço Norte) Rod. 165, Km 137, 20°19'15"S; 41°02'41"W; O. Necchi Jr., 04.vii.2009 (SJRP 31921).	KF010487	KC951863[B]	**This study**
S. delicatuliformis – BR10	Brazil: Espirito Santo, Afonso Cláudio, Comunidade de Lajinha, próximo Rod. ES-165, 20°15'01"S, 41°03'43"W; O. Necchi Jr., 04.vii.2009 (SJRP 31922).	KF010483	KC951864[B]	**This study**

Source: Author's own production

Table S5. Continued

Species/Code	Locality, collector, collection date, and herbarium voucher.	GenBank number		
		COI-5P	*rbc*L	LSU
S. delicatuliformis – BR11	Brazil: Espirito Santo, Marechal Floriano, BR-262, km 73-74, entrada para "Parque do China", 20º 24' 39''S, 40º 52' 48''W; O. Necchi Jr. 20.vii.06 (SJRP 32156).	**This study**	**This study[B]**	**This study**
S. cryptica – BR12	Brazil: Goias, Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, 14°09'58''S, 47°50'39''W; O. Necchi Jr., 06.iv.2010 (SJRP 31923).	KF010488	KC951865	**This study**
S. cryptica – BR13	Brazil: Goias, Rodovia GO-070, entre os municípios de Jussara e Itaberaí, 16º00'32,7''S, 48º55'55,9'W; F.R. Borges (SJRP 32575).	**This study**	**This study**	**This study**
S. amazonica – BR14	Brazil: Mato Grosso, Baixada Morena, Rio Roquete, BR-163, sentido Santarém, 11º32'20''S, 55º31'14''W; 19.ix.2010 (SJRP 32576).	KF010490	KC951867	**This study**
S. amazonica – BR15	Brazil: Mato Grosso, Rio Rosana, BR-163, trecho entre Sinop e Sorriso,11°57'26''S, 55°31'01''W; O. Necchi Jr., 27.ix.2010 (SJRP 31924).	KF010489	KC951866	**This study**
S. cryptica – BR16	Brazil: Mato Grosso, Sinop, BR-163, sentido Santarém; 11°36'04''S, 55°25'38'' W; O. Necchi Jr., 25.ix.2010 (SJRP 31925).	KF010491	KC951869	**This study**
S. amazonica – BR17	Brazil: Mato Grosso, Sinop, BR-163, 14 km do trevo da cidade, 12º00'37" S, 55º31"01" W; O. Necchi Jr. et al., 03.vi.2013 (SJRP 32577).	**This study**	**This study**	**This study**
S. amazonica – BR18	Brazil: Mato Grosso, Sinop, BR-163, 34 km do trevo da cidade; 11º36'05" S, 55º25'41" W; O. Necchi Jr., F.R. Borges & A.S. Garcia Fo., 04.vi.2013 (SJRP 32578).	**This study**	**This study**	**This study**
S. delicatuliformis – BR19	Brazil: Minas Gerais, Parque Nacional da Serra do Cipó, CIPÓ 3 - 25.vii.2003 (SJRP 32579).	**This study**	**This study**	**This study**
S. delicatuliformis – BR20	Brazil: Minas Gerais, Parque Nacional da Serra do Cipó, CIPÓ 8 - 07.viii.2003 (SJRP 32580).	**This study**	**This study**	**This study**
S. amazonica – BR21	Brazil, Roraima, Mucajaí, estrada para Roxinho, próximo ao trevo, 02°25'51"S, 61°02'23"W; O. Necchi Jr. et al, 05.vii.2013 (SJRP 32139 e 32140).	–	–	**This study**

Source: Author's own production

Tabela S5. Continued.

Species/Code	Locality, collector, collection date, and herbarium voucher.	GenBank number		
		COI-5P	***rbc*L**	**LSU**
North America				
S. aquiloamericana– MEX1	México: Rt. 120 in Jalpar. 24.iv.1997. No voucher specimen	**This study**	AF126414	–
S. aquiloamericana – US1	USA: Outlet canal of Montezuma Well, Arizona, 34º38'57" N, 111º45'08" W; T.A. Dempster. 24.iv.2011. (BHO A-0437, MICH 672226).	**This study**	JN408523	–
S. aquiloamericana – US2	USA: Barton Spring, Austin, Texas, 30º15'50" N, 97º46'17" W; M.L. Vis & E. Johnston. 16.xii.2010. (BHO A-0410, MICH672225)	**This study**	JF344716	–
S. delicatuliformis – CR1	Costa Rica: Rt. 16 ~8km south of San Vito. 18.ii.1998. No voucher specimen	**This study**	AF126410	–
Africa				
S. huillensis – AS1	South Africa: X31-5 No. 1, Sabie River Catchment. Gerhard Strydom. iv.2000. (BHO A-1447, MICH 672222).	MN974523	JF344717	–
S. suecica – AS2	South Africa: Tributary of the Bot River off Van der Stel Pass Road near Cape Town; 34º09'31.5" S, 19º13'33" E; M.L. Vis, W.B. Chiasson & G. Ellis. 22.viii.2005. (BHO A-	EU636738	JN408524	–
Europe				
S. suecica – FI1	Finland: Soutujoki, Housukoski, (FI-8), 62º 29' 20"N, 24º41'32" E; P. Eloranta. 12.iii.2007.	**This study**	–	**This study**
S. suecica – FI2	Finland: Meskusjoki, Kuusamo, (FI-15), 65º58'22"N, 29º08'11" E; P. Eloranta. 15.vii.2012	–	**This study**	**This study**
S. suecica – FI3	Finland: Kärkelänjoki River, Karjulankoski Rapid, (isolate 3) FI-18, 60º16'18" N, 23º35'49" E; P. Eloranta. 23.vi.2009. (MICH672231).	–	JF344722	–
S. suecica – FI4	Finland: Tarhapäänkoski, Horhanvirta River, (isolate 5) FI-22, 62º20'29" N, 24º44'05" E; P. Eloranta. 16.vii.2010 (MICH672235).	**This study**	JF344724[C]	–
S. suecica – FI5	Finland: Uittamonkoski Rapid, outlet of Lake Uittamonjärvi, (isolate 6) FI-23. P. Eloranta. 16.vii.2010. (MICH672234).	**This study**	JF344725[D]	–
S. suecica – FI6	Finland: Likojoki, Kuusamo, (FI-31), 65º47'12" N, 30º 02'52" E; P. Eloranta. 17.vii.2012.	**This study**	–	–

Source: Author's own production

Tabela S5. Continued

Species/Code	Locality, collector, collection date, and herbarium voucher.	GenBank number		
		COI-5P	***rbc*L**	**LSU**
Asia				
S. assamica – IN1a	India: Assam, Nagaon District, Chapanala; 26º19'13.7"N, 92º10'16.5" E; O. Necchi Jr., J.A. West, E.K. Ganesan & F. Yasmin, 23.ii.2018 (SJRP 32583).	MN508239	MN496129	**This study**
S. assamica – IN1b	India: Assam, Nagaon District, Chapanala; 26º19'13.7"N, 92º10'16.5"E; F. Yasmin, 25.ii.2019 (SJRP 32584)	–	–	–
S. assamica – IN2	India: Assam, Sonapur, Tegheria; 26º05'52" N, 92º02'02" E; F. Yasmin, 24.ii.2019 (SJRP 32585)	MN508240	MN496130	–
S. delicatula – MR08	Malaysia: Selangor, Tributary of Gombak River in University of Malaysia Forest Reserve, coordinates unavailable; E. Johnston, P.-E. Lim, Y. Hoi Sen, F. Saint	**This study**	KF557560	**This study**
Australasia				
S. suecica – AU1	Australia: Dom Dom Creek, Anderson Road crossing, ~1 km west of Naberthong, Victoria, 37º33'54" S, 145º39'08" E; T.J. Entwisle (3394), 05.i.2010 (NSW799516).	**This study**	JF344715	**This study**
S. suecica – AU2	Australia: Nigger Creek, Herberton, Queensland; 17º23'00" S, 145º23'00" E; T. J. Entwisle (2430), 03.vii.1994 (MEL2033246A, 2268154A).	**This study**	AF209977	**This study**
S. suecica – NZ1	New Zeland: Wanganui River, Beach track at the end of La Fontaine Road, 43º07'48" S, 170º36'37" E; M. L. Vis. 04.xii.2005 (BHO A-0265, MICH672229, WELT A027220,	–	JF344719	**This study**

Source: Author's own production

Table S6. Sequences of *rbc*L, COI-5P and LSU *Sirodotia* species obtained from Genbank used in the analyses. Superscript letters denote identical sequences.

Species	Locality, collector, collection date	GenBank Number *rbc*L COI-5P LSU	Reference
S. aquiloamericana	USA: San Marcos, San Marcos river, Texas. 1.xii.1993.	AF029157 EU636739	Vis *et al*. (1998) Sherwood *et al*. (2008)
S. kennedyi	Africa: Zambia, Mutinondo River, 12.3911S, 31.3257E, M. P. Kennedy. 07.vii.2011	MN974518	Szinte *et al* (2020)
S. suecica	USA: Rhode Island, Chipuxet River at Rt. 138, West Kingston. 27.vi.1992	AF029158	Vis *et al*. (1998)
S. suecica	USA: Town Road. in Coventry, RI. 18. Iv.1992.	AF126420	Vis & Sheath. (1999)
S. aquiloamericana	USA: New Hampshire, near Enfield, 43°38′ N, 72°09′ W.	JF701687	Necchi & Oliveira (2011)
S. delicatuliformis[a]	Brazil: Minas Gerais, Parque Nacional da Serra do Cipó, Rio Alto do Palácio, 100m da rodovia MG-010. 19º17'00"S, 43º33'00"W, 25.vii.2003.	DQ646474	Necchi *et al*. (2007)
S. delicatuliformis[a]	Brazil: São Paulo, São José do Rio Preto, Rio Lagoa, 20º49'35"S, 49º21'37"W, culture isolate.	DQ646475[A]	Necchi *et al*. (2007)
S. delicatuliformis[a]	Brazil: São Paulo, São José do Rio Preto, Rio Macaco, 20º50'31"S, 49º21'07"W, isolado de cultura.	DQ646476	Necchi *et al*. (2007)
S. delicatuliformis[a]	Brazil: São Paulo, São José do Rio Preto, Rio Talhadinho, 20º43'24"S, 49º18'21"W, 07.x.2004.	DQ646477[A]	Necchi *et al*. (2007)
S. delicatuliformis[a]	Brazil: São Paulo, Américo de Campos, Rio Aguas Paradas, 20º18'00"S, 49º46'02"W, 06.x.2004.	DQ646478[A]	Necchi *et al*. (2007)
S. suecica	USA: Canada, Labrador, 33 Km west of Goose Bay on road Churchill Falls. 30. Vii. 1993.	AF126407	Vis & Sheath. (1999)
S. suecica	USA: Big Beaver Creek, at US 176 bridge crossing, Calhoun Co., South Carolina. E.K. Hollingsworth. 16.v.2008.	JF344718 KM055337 KM055260	Lam *et al*. (2012)
S. suecica	USA: Canada, Labrador, un-named stream, 33 km west of Goose Bay on road to Churchill Falls. M. L. Vis & R. G. Sheath. 30.vii.1993.	- EU636737 -	Sherwood *et al* (2008)
S. suecica	Finland: Nytkymenjoki River, Hasakoski Rapid, (isolate 1) FI-4. P. Eloranta 20.vii.2008.	JF344720[C]	Lam *et al*. (2012)
S. suecica	Finland: Nytkymenjoki River, Hasakoski Rapid, (isolate 2) FI-10. P. Eloranta. 07.viii.2008.	JF344721	Lam *et al*. (2012)
S. suecica	Finland: Kontysjoki Upper River, (isolate 4) FI-4. P. Eloranta. 16.Jun.2010.	JF344723[D]	Lam *et al*. (2012)
S. suecica	Unpublished information	AY056021	Vis *et al*. (no date)

a. Cited as *Sirodotia delicatula.*

Source: Author's own production

Table S7. Outgroup sequences used in *rbc*L, COI-5P and LSU concatenated alignment for phylogenetic analyses.

Taxon	GenBank Number		
	***rbc*L**	**COI-5P**	**LSU**
Batrachospermales			
Acarposporophycos brasiliensis	FJ386458	KU672388	-
Batrachospermum dapsile	KM593855	-	-
Batrachospermum gelatinosum	GU810833	KM055327	KM055251
Batrachospermum gelatinosum	KJ825965	-	-
Virescentia helminthosum	AB114642	-	-
Virescentia helminthosum	AY198419	-	-
Batrachospermum naiadis	KM593857	-	-
Batrachospermum pozoazulense	KM593858	-	-
Batrachospermum shanxiense	KM593851	-	-
Batrachospermum turfosum	AY423408	-	-
Batrachospermum turfosum	AF029147	-	-
Batrachospermum viride-brasiliense	KM078048	-	-
Kumanoa ambigua	GQ368885	-	-
Kumanoa cipoensis	GQ368887	-	-
Kumanoa gracilima	JN590013	-	-
Kumanoa holtonii	JN590004	JN604921	
Kumanoa louisianae	JN590005	JN604924	
Kumanoa procarpa	FJ386464	KM055332	KM055256
Lemanea borealis	AF029149	-	-
Lemanea fluviatilis	KM055243	KM055333	KM055257
Lemanea fucina	KJ825958	KU672391	-
Lympha mucosa	KM593865	KM593873	-
Montagnia australis	EU106056	KT802766	
Montagnia macrospora	EU106060	KU672389	
Montagnia macrospora	AY423406	-	-

Source: Author's own production

Table S7. Continued.

Taxon	GenBank Number		
	***rbc*L**	**COI-5P**	**LSU**
Nocturama antipodites	FJ386456	KT802754	-
Nocturama antipodites	KT802838	-	-
Nothocladus diatyches	KT802848	KT802758	-
Nothocladus theaquus	KT802863	KT802763	-
Paludicola keratophyta	KJ825960	MN943976	-
Paludicola turfosa	MN943940	MN943985	-
Paralemanea annulata	GQ285124	KM055335	KM055259
Paralemanea catenata	AF029154	-	-
Petrohua bernabei	AY960690	KM055336	AY960689
Psilosiphon scoparium	AF029155	KU672392	-
Sheathia americana	JX669757	JX669695	KM055247
Sheathia arcuata	KM593811	KM592946	-
Sheathia confusa	DQ393133	JX669703	KM055250
Sheathia confusa	JX669737	JX669683	-
Sheathia exígua	JX669738	-	-
Sheathia involuta	KU672395	KU672393	-
Torularia atra	KT183025	KT894750	-
Torularia puiggariana	KT183015	KT894740	-
Tuomeya americana	KM055244	KM055330	KM055254
Virescentia viride-americana	AF244112	KM055328	-
Virescentia viride-brasiliensis	KM078048	KM259993	-
Visia cayennensis	AY423393	KM055326	KM055249
Visia sp.	KM055245	KM055325	KM055248
Visia cylindrocelularis	KF557568	-	-
Volatus carrrioni	KM593861	KM593866	-
Volatus ulterior	KM593852	-	-
Volatus personatus	KM593856	-	-
Thoreales			
Thorea hispida	GU169076	-	-
Thorea reikei	KM005140	KM055239	-

Source: Author's own production

Table S8. Morphological characters of *Sirodotia* specimens examined in this study or reported in relevant works. Sample code according to Table S5. For numerical data, the values (µm) represent the amplitude of variation (upper line) and mean ± standard deviation (lower line)

Species	Separation of sex	Whorl		Internode length	Primary fascicles						
					number of cells	Proximal		Distal			
		Diameter	Arrange			Length	Diameter	Length	Diameter	Shape	L/D
S. acuminata Necchi *et al*. (1993)	Monoecious	317-497 (391)	Dense	–	6-9 (7.3)	Cylindrical, Obovoidal		–	–	Ellipsoidal, Obovoidal	–
S. amazonica This study (31924, 32139, 32140, 32576)	Monoecious, Dioecious	249-491 (378 ± 60)	Dense, Open	455-1351 (859 ± 271)	4-8 (6.0 ± 1.1)	Cylindrical, Obovoidal, 18-40 (29.3 ± 6.4)	Cylindrical, Obovoidal, 9-17 (11.1 ± 1.7)	9-16 (11.1 ± 1.9)	7-15 (9.3 ± 1.9)	Spherical, Subspherical, Ellipsoidal	1.0-1.6 (1.2 ± 0.2)
S. aquiloamericana This study (US1, US2)	Dioecious	408-675 (566 ± 70)	Open	542-1108 (822 ± 188)	7-12 (8.8 ± 1.4)	Cylindrical, Obovoidal, 22-37 (28.9 ± 3.7)	Cylindrical, Obovoidal, 8-17 (11.9 ± 2.6)	10-15 (12.6 ± 1.5	6-12 (8.8 ± 1.9)	Subspherical, Ellipsoidal, Obovoidal	1.2-1.8 (1.5 ± 0.3)
Necchi *et al.* (1993)	Dioecious	238-430 (307)	–	–	7-10 (7.9)	–	–	–	–	–	–
S. assamica Rossignolo *et al.* (2020)	Dioecious (Masculine), Monoecious	400-665 (495 ± 76)	Dense, Open	547-1177 (887 ± 182)	6-12 (8.6 ± 1.4)	Cylindrical, Ellipsoidal 17-42 (25.8 ± 4.7)	Cylindrical, Ellipsoidal 7-13 (8.7 ± 1.1)	8-15 (11.6 ± 1.4)	5-9 (6.7 ± 0.8)	Ellipsoidal, Obovoidal	1.2-2.2 (1.7 ± 0.2)
S. ateleia *Necchi et al.* (1993)	Monoecious, Dioecious	193-484 (263)	–	–	6-10 (8.2)	–	–	–	–	–	–
Skuja (1938)	Dioecious	360	–	–	5-8	Cylindrical, Ellipsoidal, 23 x 11		14-28	14	Subspherical, Obovoidal	–
S. cirrhosa Balakrishnan & Chaugule (1980)	Dioecious	95-540	–	–	5-9	Ellipsoidal, Ovoid 16-23	Ellipsoidal, Ovoid 6.5-12	–	–	–	–
S. cryptica This study (31923, 31925, 32575)	Monoecious, Dioecious	223-559 (359 ± 80)	Dense	200-1079 (468 ± 160)	4-11 (7.0 ± 1.0)	Cylindrical, Ellipsoidal 20-44 (29.6 ± 6.2)	Cylindrical, Ellipsoidal 8-19 (11.7 ± 1.9)	9-17 (12.1 ± 1.7)	7-13 (9.6 ± 1.2)	Spherical, Ellipsoidal, Obovoidal	1.0-1.6 (1.3 ± 0.2)
S. delicatula This study (MR08)	Monoecious	220-433 (296 ± 71)	Dense	248-500 (409 ± 79)	6-8 (7.0 ± 0.7)	Cylindrical, Ellipsoidal 21-44 (30.7 ± 6.3)	Cylindrical, Ellipsoidal 8-13 (11.3 ± 1.6)	10-14 (12.4 ± 1.2)	7-11 (9.4 ± 1.2)	Subspherical, Obovoidal	1.1-1.8 (1.3 ± 0.2)
Skuja (1938)	Monoecious	260	Dense	–	–	Cylindrical, 14-21	Cylindrical, 7-11	12-18	7-12	Obovoidal	–
Umezaki (1960)	Dioecious	–	–	–	–	Ellipsoidal, Obovoidal, 9-21 x 4-11					–
Necchi *et al*. (1993)	Monoecious	137-306 (188)	–	–	5-8 (6.6)	–	–	–	–	–	–

Source: Author's own production

Table S8. Continued

Species	Separation of sex	Whorl		Internode length	Primary fascicles						
					number of cells	Proximal		Distal			
		Diameter	Arrange			Length	Diameter	Length	Diameter	Shape	L/D
S. delicatuliformis This study (31917, 31918, 31919, 23450, 31920, 31921, 31922)	Monoecious Dioecious, Polioecious	169-491 (362 ± 64)	Dense, Open	242-843 (530 ± 147)	5-13 (7.0 ± 1.6)	Cylindrical, Ellipsoidal, Obovoidal 17-44 (30.4 ± 8.0)	Cylindrical, Ellipsoidal, Obovoidal 8-18 (11.9 ± 2.4)	8-16 (11.8 ± 1.6)	5-14 (8.9 ± 1.7)	Spherical, Ellipsoidal, Obovoidal	1.0-2.0 (1.3 ± 0.2)
S. fennica Lectotype	Dioecious	217-230[a] (224 ± 5)	Open	209-360[a] (275 ± 67)	7-9[a] (8 ± 0.7)	Cylindrical, Obovoidal 16-22a (18 ± 2,1)	Cylindrical, Obovoidal, 7-8a (7 ± 0,4)	8-11[a] (9 ± 1.5)	5-7[a] (6 ± 0.9)	Ellipsoidal, Obovoidal	1.5-1.7 (1.6 ± 0.1)
Skuja (1931)	Monoecious	400	–	–	–	Cylindrical, Obovoidal	Cylindrical, Obovoidal	–	–	Obovoidal, Ellipsoidal	–
Necchi *et al.* (1993)	Monoecious	229-341	–	–	6-9 (7.4)	–	–	–	–	–	–
S. gardneri Necchi *et. al.* (1993)	Dioecious	288-463 (384)	–	–	6-10 (8.3)	–	–	–	–	–	–
S. goebelii Entwisle & Foard (1999)	Monoecious	230-370	Dense	368-529	9-12	Ellipsoidal, 18-32	Ellipsoidal, 8-10	6-15	3-7	–	–
S. huangshanensis Z. X. Shi et S. L. Xie (2004).	Monoecious	380-430	Dense	–	6-9	Cylindrical, Ellipsoidal, 14-25	Cylindrical, Ellipsoidal, 7-8	–	–	–	–
S. huillensis This study (AS1)	Monoecious, Dioecious	234-345 (265 ± 29)	Dense	200-450 (309 ± 67)	5-7 (6.0 ± 0.8)	Cylindrical, Ellipsoidal, 11-15 (12.2 ± 1.2)	Cylindrical, Ellipsoidal, 7-9 (7.6 ± 0.5)	8-11 (9.6 ± 1.1)	6-9 (7.3 ± 1.1)	Subspherical Obovoidal	1,1-1,6 (1.3 ± 0.2)
S. huillensis Isotype	Monoecious	224-330 (256 ± 31)	Open	470-877 (669 ± 186)	7-8 (7.4 ± 0.5)	Cylindrical, Ellipsoidal, Obovoidal 16-22 (19.5 ± 5.5)	Cylindrical, Ellipsoidal, Obovoidal 10-14 (12.0 ± 3.5)	9-15 (12.1 ± 3.7)	7-12 (9.4 ± 3.1)	Subspherical Obovoidal	1,0-1,6 (1.3 ± 0.2)
Skuja (1931)	Monoecious	234-364 (278 ± 79)	Open	195-558 (285 ± 112)	–	Cylindrical, Obovoidal, 15-25	Cylindrical, Obovoidal, 5-14	–	–	Ellisoidal, Obovoidal	–
Necchi *et al*. (1993)	Monoecious	162-278 (214)	–	–	6-10 (7.4)	–	–	–	–	–	–

Source: Author's own production

Table S8. Continued

Species	Separation of sex	Whorl		Internode length	Primary fascicles						
					number of cells	Proximal		Distal			
		Diameter	Arrange			Length	Diameter	Length	Diameter	Shape	L/D
S. kennedyi Szinte *et al.* (2020)	Dioecious	198-315	–	82-90	3-5	Cylindrical, Ellipsoidal, Obovoidal					–
S. segawae Isotype	Dioecious	235-500 (320 ± 76)	Dense	487-650 (559 ± 47)	7-10 (8.3 ± 1.1)	Ellipsoidal, Obovoidal 20-37 (23 ± 4.6)	Ellipsoidal, Obovoidal 8-14 (11 ± 1.6)	9-16 (12.8 ± 1.9)	7-10 (8.0 ± 0.9)	Ellipsoidal, Obovoidal	1.4-2.0 (1.6 ± 0.3)
S. sinica Jao (1941)	Monoecious	300-370	–	–	7-10	Ovoidal					–
S. suecica This study (FI1, FI2, FI6, AS2, NZ1)	Monoecious, Dioecious	209-461 (327 ± 67)	Dense	217 - 715 (454 ± 117)	5-11 (7.4 ± 1.4)	Cylindrical, Obovoidal 17-34 (24.0 ± 5.0)	Cylindrical, Obovoidal 7-17 (11.2 ± 2.7)	9-15 (11.5 ± 1.6)	6-12 (8.4 ± 1.7)	Subspherical Ellipsoidal, Obovoidal	1.0-2.0 (1.5 ± 0.3)
Isotype	Dioecious	200-352 (257 ± 38)	Dense	487-710 (590 ± 53)	7-10 (8.9 ± 1.2)	Cylindrical, Obovoidal 20-32 (24.1 ± 3.6)	Cylindrical, Obovoidal 8-13 (9.2 ± 1.5)	10-14 (11.4 ± 1.2)	6-10 (7.0 ± 1.2)	Obovoidal, Ellipsoidal	1,3-2,0 (1.7 ± 0.2)
Israelson (1942)	Monoecious	(110-) 200-300 (-500)	–	(180-) 300-650 (-940)	(5-)7-8 (-10)	Cylindrical, Ellipsoidal, Obovoidal 12-30	Cylindrical, Ellipsoidal, Obovoidal 6-8(-10)	–	–	–	–
Entwisle & Kraft (1984)	Monoecious	150-350	Dense	150-800	4-10	Cylindrical, Ellipsoidal, Obovoidal 10-30	Cylindrical, Ellipsoidal, Obovoidal 2-7	7-13	3-6	–	–
Necchi *et al.* (1993)	–	229 - 662	Dense	–	5-10	–	–	–	–	–	–
Entwisle & Foard (1999)	Monoecious, Dioecious	(85-) 135-850	Dense	135-875	4-10 (-13)	Cylindrical, Ellipsoidal, Fusiform, Obovoidal 10-56	Cylindrical, Ellipsoidal, Fusiform, Obovoidal 6-10	6-15(-18)	(2.5-)4-8	Subspherical	–
Eloranta *et. al.* (2011)	Monoecious	200-300	Dense	–	–	–	–	–	–	–	–
S. tenuissima Necchi *et al.* (1993)	Monoecious	191-628 (373)	Dense	–	5-10 (7.2)	–	–	–	–	–	–
Eloranta *et al.* (2011)	Dioecious	–	Dense	–	–	–	–	–	–	–	–
S. yutakae Isotype	Monoecious	157-282 (214 ± 43)	Open	415-643 (581 ± 70)	6-10 (8.3 ± 1.1)	Ovoidal, Obovoidal 19-35 (23.0 ± 4.2)	Ovoidal, Obovoidal 8-15 (11.5 ± 2.0)	9-14 (12.0 ±1.6)	7-10 (8.1 ± 0.8)	Obovoidal Ellipsoidal	1.2-2.0 (1.5 ± 0.2)

Source: Author's own production.

Table S9. Reproductive characters of *Sirodotia* specimens examined in this study or reported in relevant works. Sample code as Table S5. For numerical data the values (µm) represent amplitude of variation (upper line) and mean ± standard deviation (lower line).

Species	Carpogonium			Number of cells in the carpogonial branch	Carposporangia			Number of erect gonimoblast filament cells	Spermatangia	
	Length	Diameter	Shape		Length	Diameter	Shape		Diameter	Shape
S. acuminata Necchi *et al.* (1993)	19-37 (28.1)	5-9 (6.1)	Elongate cylindrical, ellipsoidal or lageniform	–	–	–	–	–	5-7 (6.3)	Spherical
S. amazonica This study (31924, 32139, 32140, 32576)	35-62 (44.3 ± 6.8)	8-15 (11.1 ± 1.6)	Elongate cylindrical (wavy margins) or Fusiform	1-4 (2.5 ± 1.3)	10-19 (13.6 ± 1.9)	8-13 (10.1 ± 1.2)	Obovoidal, Subspherical	1-4 (2.5 ± 0.9)	6-9 (7.6 ± 0.7)	Spherical, Obovoidal
S. aquiloamericana This study (US1, US2)	–	–	–	–	10-13 (11.0 ± 0.8)	6-8 (6.7 ± 0.7)	Obovoidal	2-5 (3.6 ± 0.9)	5-8 (6.4 ± 0.6)	Spherical, Obovoidal
Necchi *et al.* (1993)	29-42 (35)	6-7.5 (6.8)	Elongate cylindrical (with wavy margins) or fusiform	–	–	–	Subspherical	–	5-7 (6.0)	Spherical
S. assamica Rossignolo *et al.* (2020)	37-64 (51.7 ± 7.3)	10-16 (11.6 ± 1.4)	Elongate cylindrical, ellipsoidal or lageniform	1-5 (2.6 ± 1.3)	11-14 (12.2 ± 0.8)	6-8 (7.2 ± 0.1)	Obovoidal	1-4 (2.4 ± 0.8)	6-8 (7.0 ± 0.6)	Spherical
S. ateleia *Necchi et al. (1993)*	42-65 (54.4)	5-8 (6.6)	Elongate cylindrical	–	–	–	–	–	–	–
Skuja (1938)	45-70	–	Elongate cylindrical, fusiform, club-shaped	2-4	10-13[3]	6-8[3]	Obovoidal	–	5-7[3]	Spherical
S. cirrhosa Balakrishnan & Chaugule (1980)	29-41	8-10	Elongate cylindrical	3-5	8-11	5.5-8	Ellipsoidal, Obovoidal	–	6.5-7.5	Subspherical
S. cryptica This study (31923, 31925, 32575)	24-58 (42.7 ± 8.3)	9-15 (10.5 ± 1.4)	Elongate cylindrical (with wavy margins) or fusiform	1-4 (1.7 ± 1.0)	10-17 (12 ± 2.2)	8-11 (9.4 ± 0.9)	Subspherical, Ellipsoidal, Obovoidal	2-5 (3.6 ± 1.1)	6-9 (7.9 ± 0.8)	Spherical Obovoidal

3. According Kumano (1982).

Source: Author's own production

Table S9. Continued

Species	Carpogonium			Number of cells in the carpogonial branch	Carposporangia			Number of erect gonimoblast filament cells	Spermatangia	
	Length	Diameter	Shape		Length	Diameter	Shape		Diameter	Shape
S. delicatula This study (MR08)	30-57 (38.5 ± 8.7)	8-13 (10.4 ± 1.2)	Elongate cylindrical, Fusiform, Ellipsoidal, wavy margins	2-4 (3.0 ± 0.8)	10-16 (11.7 ± 2.1)	6-10 (7.6 ± 1.4)	Obovoidal	1-3 (2.1 ± 0.8)	7-8 (7.3 ± 0.5)	Spherical
Skuja (1938)	24-41	5.5-7	Cylindrical	2-3	14	7	Obovoidal	–	7-8	Ovoidal
Umezaki (1960)	19-34	5-7	Cylindrical, Club-shaped	3-7	8-11	5-7	Obovoidal	2-3	4-7	Spherical
Necchi *et al.* (1993)	25-40 (30.3)	5,5-7 (6.3)	Elongate cylindrical, Fusiform, Ellipsoidal	3-5	–	–	–	–	7-8 (7.4)	Spherical
S. delicatuliformis This study (31917, 31918, 31919, 23450, 31920, 31921, 31922)	20-59 (38.7 ± 8.1)	8-16 (10.4 ± 1.7)	Elongate cylindrical, Fusiform, wavy margins	0-4 (1.3 ± 0.9)	11-16 (13.2 ± 1.2)	6-9.5 (7.4 ± 0.8)	Obovoidal, Ellipsoidal	1-4 (2.3 ± 1.0)	5.0-8.5 (6.3 ± 1.0)	Spherical, Obovoidal
S. fennica Lectotype	–	–	–	–	–	–	–	–	5-6 (5 ± 0.5)	Spherical
Skuja (1931)	18-25	9	Elongate cylindrical	3-5(-6)	–	–	–	–	5-6	Spherical
Necchi *et al.* (1993)	18-27 (22.5)	4.5-6 (5.1)	–	–	–	–	–	–	4-7 (5.4)	Spherical
S. gardneri Necchi *et. al.* (1993)	20	–	Elongate cylindrical	–	–	–	–	2-3	5-8 (6,4)	Spherical
S. goebelii Entwisle & Foard (1999)	26-38	11-16	Ellipsoidal, Cylindrical	2-6	13-21	6-9	Ellipsoidal	5-9	5-9	Spherical
S. huangshanensis Z. X. Shi & S. L. Xie (2004)	48-55	10	Elongate cylindrical, Ellipsoidal	2-7	9-12	7-10	Ellipsoidal	3-4	6-8	Spherical

Source: Author's own production

Table S9. Continued

Species	Carpogonium			Number of cells in the carpogonial branch	Carposporangia			Number of erect gonimoblast filament cells	Spermatangia	
	Length	Diameter	Shape		Length	Diameter	Shape		Diameter	Shape
S. huillensis This study (AS1)	34-42[2]	–	Elongate cylindrical, wavy margins	1-3[2]	8[a] (8.3 ± 0.1)	5-6[a] (5.9 ± 0.4)	Obovoidal	2-4[a] (3.0 ± 0.8)	5-7[a] (5.9 ± 0.7)	Spherical
S. huillensis Isotype	–	–	–	–	–	–	–	–	5-6.5[a] (5.5 ± 0.6)	Spherical
Skuja (1931)	32-48	10-12	Elongate cylindrical, Fusiform, wavy margins	1-3	7-10	4-8	Obovoidal	2-3	6-7	Spherical
Necchi *et al*. (1993)	28.5-47	5.5-7.5	Elongate cylindrical	–	–	–	–	–	6-7	Spherical
S. kennedy Szinte *et al.* (2020)	25-40	–	Elongate cylindrical, club-shaped	3-4	9-10	6-7	Obovoidal	1-2	–	–
S. segawae Isotype	30-46[3]	–	Elongate cylindrical	3-9[3]	10-20[a] (14.3 ± 3.8)	8-11[a] (9.8 ± 1.1)	Ovoidal, Obovoidal	3-4 (3.3 ± 0.4)	6-8[3]	Spherical[3]
S. sinica Jao (1941)	36 - 41	5.4-6.3	Elongate cylindrical, club-shaped	4-8	12-18	9-10	Subspherical	–	7-9	Spherical

a. N= 2-5; 1. According Flint (1950); 2. According Lam *et al.* (2012); 3. According Kumano (1982).

Source: Author's own production

Table S9. Continued

Species	Carpogonium			Number of cells in the carpogonial branch	Carposporangia			Number of erect gonimoblast filament cells	Spermatangia	
	Length	Diameter	Shape		Length	Diameter	Shape		Diameter	Shape
S. suecica This study (FI1, FI2, FI6, AS2, NZ1)	24-39 (28.5 ± 3.7)	8-15 (10.7 ± 2.1)	Elongate cylindrical, Fusiform, wavy margins, Ellipsoidal	1-5 (2.5 ± 1.2)	10-13 (10.7 ± 0.9)	6-11 (8.0 ± 1.1)	Obovoidal	2-5 (3.2 ± 0.7)	5-8 (6.5 ± 0.8)	Spherical
Isotype	24-25[a] (24.5 ± 0.5)	10-11[a] (10.5 ± 0.5)	Elongate cylindrical	2-3[a] (2.5 ± 0.5)	11-12[a] (11.7 ± 0.4)	7-9[a] (7.8 ± 0.5)	Obovoidal	2-3[a] (2.8 ± 0.4)	–	–
Israelson (1942)	20-26	–	Cylindrical	2-5	8-12	6-8	Obovoidal	–	6-7.5	Spherical, Ellipsoidal
Entwisle & Kraft (1984)	19-42	4-8	Cylindrical, Ellipsoidal, Pear-shaped	3-5	8-10	6-8	Ellipsoidal, Pear-shaped	3-5	4-5.5	Spherical
Necchi *et al.* (1993)	19-42	4-8	Cylindrical	–	–	–	–	–	5-8	–
Entwisle & Foard (1999)	15-42	7-11	Cylindrical, Fusiform	2-7	8-13(-17)	6-9	Obovoidal, Ellipsoidal, Spherical	2-5(-7)	4-8	Spherical
Eloranta *et al.* (2011)	30-40	–	Cylindrical, Ellipsoidal, Pear-shaped	2-5	8-12	6-8	Obovoidal	2-3	5-6	Spherical
S. tenuissima Necchi *et al.* (1993)	20.5-37 (27.8)	5-9.5 (6.5)	Ellipsoidal, Pear-shaped	–	–	–	–	–	5-8.5 (6.3)	Spherical
Eloranta *et al.* (2011)	30-40	–	Ellipsoidal Cylindrical, Pear-shaped	2-5	–	–	Ovoidal	2-3	–	–
S. yutakae Isotype	20-48[a] (33 ± 10.9)	9-11[a] (9.9 ± 0.6)	Cylindrical, Pear-shaped	2-4[a] (3.3 ± 0.8)	10-14[a] (11.4 ± 1.6)	8-10[a] (8.9 ± 0.6)	Obovoidal	2-3[a] (2.5 ± 0.5)	5-8[a] (6.3 ± 1.1)	Spherical

a. N= 2-5.

Source: Author's own production

Table S10. Comparative morphological characters and geographic distribution of some species of *Sirodotia*. The dimension data are presented in µm.

Species	Whorls diameter	Primary fascicle cell number	Shape, Length / diameter of distal cells of primary fascicles	Diameter and arrangement of spermatangia	Carpogonial branch length	Carpogonium length	Origin of gonimoblast filaments	Shape and size of carposporangia		Geographic distribution	References
								Length	Diameter		
S. acuminata	317-497 (391)	6-9 (7.3)	Ellipsoidal, Obovoidal	Terminal, in pairs, 5-7 (6.3)	–	19-37 (28.1)	on the opposite side of the basal protuberance	–	–	North America: USA	Flint (1950), Necchi *et al.* (1993)
S. amazonica	249-491 (378 ± 60)	4-8 (6.0 ± 1.1)	Spherical, Subspherical, Ellipsoidal; 0.8-1.6(-1.7) (1.2 ± 0.2)	Terminal or subterminal, single or in pairs, 6-9 (7.6 ± 0.7)	8.5-25 (16 ± 5.6)	35-58(-62) (44.5 ± 6.8)	basal protuberance	Obovoidal, Ovoidal, Subspherical, 10-18(-19) (13.6 ± 1.9)	8-13 (10.1 ± 1.2)	South America: Brazil	This study
S. aquiloamericana	408-675 (566 ± 70)	7-12 (8.8 ± 1.4)	Subspherical, Ellipsoidal, Obovoidal; 1.0-2.1 (-2.5) (1.5 ± 0.3)	Terminal, single or in pairs, 5-7(-8) (6.1 ± 0.6)	–	–	–	Obovoidal, 10-13 (11.0 ± 0.8)	6-8 (6.7 ± 0.7)	North America: Mexico, USA	This study
S. assamica	400-665 (495 ± 76)	6-11(-12) (8.6 ± 1.4)	Ellipsoidal, Obovoidal; (1.2-)1.3-2.1(-2.2) (1.7 ± 0.2)	terminal or sub-terminal, in clusters, 6-8 (7.0 ± 0.6)	7-23 (16 ± 4.3)	37-64 (51.7 ± 7.3)	basal protuberance	Obovoidal, 11-14 (12.2 ± 0.8)	6-8 (7.2 ± 0.1)	Asia: India	Rossignolo *et al.* (2020)
S. ateleia	193-484	5-10	Subspherical, Obovoidal	Terminal, single, 5-7	–	42-70	basal protuberance	–	–	Asia: Indonesia and Malaysia	Skuja (1938), Necchi *et al*. (1993)
S. cirrhosa	95-540	5-9	–	Terminal, single or in pairs, 6-7	20-30[1] (24.3 ± 4.1)	29-41	basal protuberance	Ellipsoidal, Obovoidal 8-11	5.5-8	Asia: India	Balakrishnan & Chaugule (1980)
S. cryptica	223-559 (359 ± 80)	(4-)5-10(-11) (7.0 ± 1.3)	Spherical, Ellipsoidal, Obovoidal; 1.0-1.6 (1.3 ± 0.2)	Terminal or subterminal, single or in pairs, 6-9 (7.9 ± 0.8) 10-25 (16.3 ± 5.3)	10-25 (16.3 ± 5.3)	(24-) 26-58 (42.7 ± 8.3)	basal protuberance	Obovoidal, Ellipsoidal, Subspherical, 10-17 (12.0 ± 2.2)	8-11 (9.4 ± 0.9)	South America: Brazil	This study
S. delicatula	137-433 (296 ± 71)	(-5)6-8 (7.0 ± 0.7)	Subspherical, Obovoidal; 1.1-1.7(-1.8) (1.3 ± 0.2)	Terminal, single or in pairs, (4-)7-8 (7.3 ± 0.5)	8-17(-20) (12.3 ± 2.2)	(19-)24-57 (38.5 ± 8.7)	basal protuberance	Obovoidal 10-16 (11.7± 2.1)	5-10 (7.6 ± 1.4)	Asia: Indonesia, Japan and Malaysia	Skuja (1938), Umezaki (1960), Necchi *et al.* (1993), This study

1. Data extracted from the figures of the original works.

Table S10. Continued

Species	Whorls diameter	Primary fascicle cell number	Shape, Length / diameter of distal cells of primary fascicles	Diameter and arrangement of spermatangia	Carpogonial branch length	Carpogonium length	Origin of gonimoblast filaments	Shape and size of carposporangia		Geographic distribution	References
								Length	Diameter		
S. delicatuliformis	169-491 (362 ± 64)	5-10 (-13) (7.0 ± 1.6)	Spherical, Ellipsoidal, Obovoidal; 1.0-1.7(-2.0) (1.3 ± 0.2)	Terminal or subterminal, single or in pairs; 5-8(-8.5) (6.3 ± 1.0)	6-22 (13.7 ± 5.1) (20-)	(20-) 22-55(-59) (38.7 ± 8.1)	basal protuberance	Obovoidal, Ovoidal, Ellipsoidal, 11-16 (13.2 ± 1.2)	6-8,5(-9.5) (7.4 ± 0.8)	South America: Brazil; Central America: Costa Rica	This study
S. fennica	217-400 (224 ± 5)	6-9 (8 ± 0.7)	Ellipsoidal, Obovoidal; 1.5-1.7 (1.6 ± 0.1)	Terminal, single or in pairs, 4-7 (5 ± 0.5)	–	18-27 (22.5)	on the opposite side of the basal protuberance	–	–	Europe	Skuja, (1931), Necchi *et al*. (1993), This study
S. gardneri	288-463	6-10	–	Terminal, single or in pairs, 5-8	–	20	–	–	–	North America: USA	Flint (1950), Necchi *et al.* (1993)
S. goebelii	230-370	9-12	–	Terminal, 5-9	–	26-38	on the opposite side of the basal protuberance	Ellipsoidal 13-21	6-9	Australia and New Zealand	Entwisle & Foard (1999)
S. huangshanensis	380-430	6-9	–	Terminal, single, in pairs, or in clusters, 6-8	10-45[1] (21.3 ± 12.6)	48-55	basal protuberance	Ellipsoidal, 9-12	7-10	Asia: China	Xie & Shi (2004), Hu & Wei (2006)
S. huillensis	162-345 (260 ± 30)	5-9(-10) (7.0 ± 1.0)	Subspherical, Ellipsoidal, Obovoidal 1.0-1.7 (1.3 ± 0.2)	Terminal, single or in pairs, 5-7 (5.9 ± 0.7)	5-14[1] (9 ± 3.2)	28.5-48	basal protuberance	Ovoidal, Obovoidal 8-10 (8.3 ± 0.4)	5-8 (5.9 ± 0.4)	Africa: South Africa	Necchi *et al.* (1993), Lam *et al.* (2012), This study
S. kennedyi	198-315	3-5	Ellipsoidal, Obovoidal	–	16-22[1] (19 ± 2.8)	25-40	–	Obovoidal, 9-10	6-7	Africa: South Africa	Szinte *et al*. (2020)

1. Data extracted from the figures of the original works.

Source: Author's own production

Table S10. Continued

Species	Whorls diameter	Primary fascicle cell number	Shape, Length / diameter of distal cells of primary fascicles	Diameter and arrangement of spermatangia	Carpogonial branch length	Carpogonium length	Origin of gonimoblast filaments	Shape and size of carposporangia		Geographic distribution	References
								Length	Diameter		
S. segawae	235-500 (320 ± 76)	7-10 (8.3 ± 1.1)	Ellipsoidal, Obovoidal; 1,1-2,0 (1.6 ± 0.3)	Terminal, specialized branches, 6-8	20-40[1] (31.4 ± 7.2)	30-46	on the opposite side of the basal protuberance	Obovoidal, Ovoidal, 10-20 (14.3 ± 3.8)	8-11 (9.8 ± 1.1)	Asia: Japan	Kumano (1982), This study
S. sinica	300-370	7-10	–	Terminal, single, 7-9	–	36-41	basal protuberance	Subspherical 12-18	9-10	Asia: China	Jao (1941)
S. suecica	(85-) 135-850 (315 ± 73)	4-11(-13) (7.6 ± 1.7)	Spherical, Ellipsoidal, Obovoidal; 0.9-2.0 (1.5 ± 0.3)	Terminal, single or in pairs, (4-)5-8 (6.6 ± 0.8)	5-20 (12.7 ± 4.8)	(15-)19-42 (28.5 ± 3.7)	on the opposite side of the basal protuberance	Obovoidal, Ellipsoidal, Piriform, Spherical 10-13 (10.7 ± 0.9)	6-10(-11) (8.0 ± 1.1)	Europe, North America, Africa, Asia, Australasia	Israelson (1942), Entwisle & Kraft (1984), Necchi *et al*. (1993), Entwisle & Foard (1999), Eloranta *et al*. (2011), This study
S. tenuissima	191-628 (373)	5-10 (7.2)	–	–	–	30-40	on the opposite side of the basal protuberance	Ovoidal	–	North America and Europe	Necchi *et al*. (1993), Eloranta *et al*. (2011)
S. yutakae	157-282 (214 ± 43)	6-10 (8.3 ± 1.1)	Obovoidal, Ellipsoidal; 1.2-1.7(-2.0) (1.5 ± 0.2)	Terminal, specialized branches, 5-8 (6.3 ± 1.1)	10-20[1] (14.1 ± 3.3)	20-48 (33 ± 10.9)	on the opposite side of the basal protuberance	Ovoidal, Obovoidal, 10-14 (11.4± 1.6)	8-10 (8.9 ± 0.6)	Asia: Japan	Kumano (1982), This study

1. Data extracted from the figures of the original works.

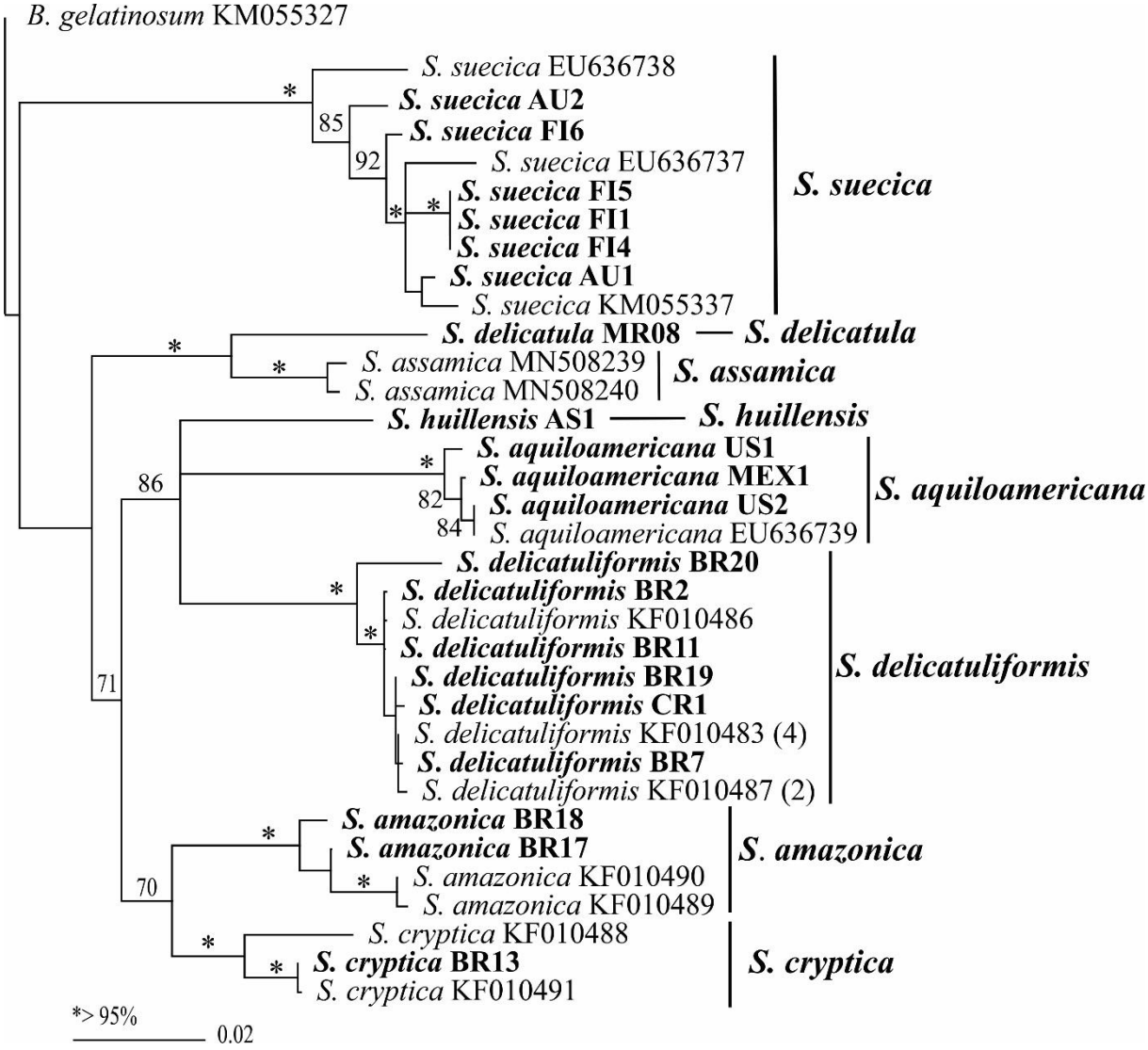
Source: Author's own production

Fig. S1. Maximum likelihood phylogenetic tree based on *rbc*L. The numbers associated with the nodes indicate the bootstrap values (BS) for maximum likelihood and posterior probability (PP) for Bayesian analysis; nodes without values indicate BS ≤ 70% and PP≤ 0.70. Sample codes as



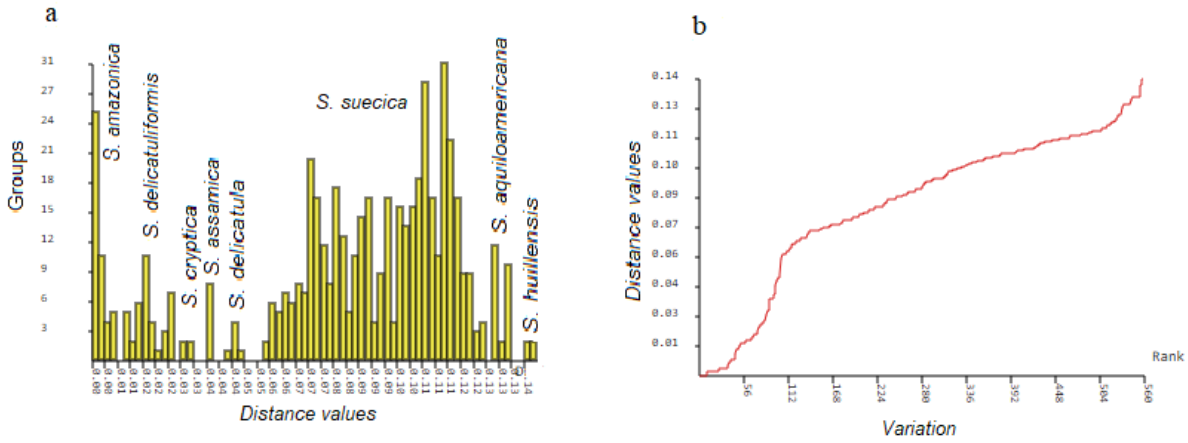
Source: Author's own production

Fig. S2. Distance tree (neighbor-joining) based on COI-5P sequences. * = bootstrap values > 95; nodes without values indicate bootstrap values ≤ 70. Information on the sequences are listed in Table S5-S6 (Supporting Information). Scale represents substitutions per site.



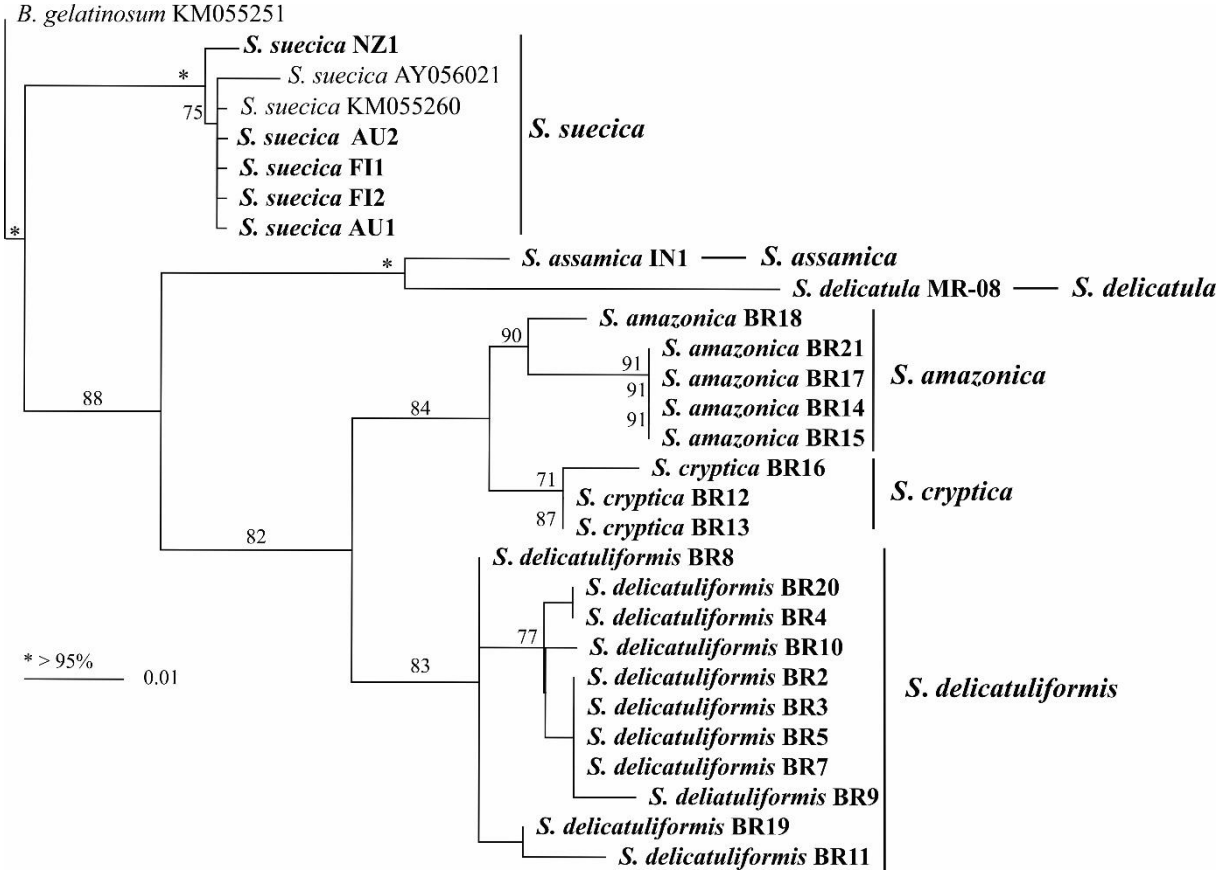
Source: Author's own production

Fig. S3. Graphical representation of the results of Automatic Barcode Gap Discovery (ABGD) for the COI-5P sequences of the *Sirodotia* genus used in this study. a. Histogram of the distribution of the different distance values for each species. B. The same distance data represented as ordered values.



Source: Author's own production

Fig. S4. Distance tree (neighbor-joining) based on LSU sequences. * = bootstrap values > 95; nodes without values indicate bootstrap values ≤ 70. Information on the sequences are listed in Table S5-S6 (Supporting Information). Scale represents substitutions per site.



Source: Author's own production

4. CONCLUSÕES GERAIS

Os resultados obtidos no presente trabalho confirmaram o gênero *Sirodotia* como um grupo monofilético com alto suporte dentro da ordem Batrachospermales, conforme reportado em estudos filogenéticos anteriores (VIS *et al*., 1998; VIS & SHEATH, 1999; NECCHI *et al*., 2007; ENTWISLE *et al*., 2009; PAIANO & NECCHI, 2013). Este resultado corrobora a primeira hipótese de que *Sirodotia* consiste em um grupo monofilético dentro da ordem Batrachospermales e que deve ser mantido como gênero.

Com relação à diversidade de espécies, dos 23 nomes de espécies listadas no gênero antes deste estudo (Ott, 2009; Guiry & Guiry, 2020) (Tabela 4), três espécies aceitas taxonomicamente não puderam ser reavaliadas (*S. cirrhosa*, *S. gardneri* e *S. huangshanensis*), pois os espécimes tipo não estavam disponíveis para análise e os caracteres utilizados não foram considerados taxonomicamente informativos. Quatro espécies (*S. iyengarii, S. polygama, S. angolensis* e *S. nigrescens*) foram removidas de *Sirodotia* anteriormente ou neste estudo por apresentarem características de outros gêneros de Batrachospermales. Quatro espécies foram sinonimizadas neste trabalho, três delas (*S. segawae*, *S. sinica* e *S. yutakae*) com *S. suecica* e *S. ateleia* foi sinonimizada com *S. delicatula*. Quatro espécies (*S. acuminata*, *S. fennica*, *S. goebelii* e *S. tenuissima*) já haviam sido sinonimizadas anteriormente com *S. suecica*. Outros três nomes (*S. ambigua*, *S. loefgrenii* e *S. moniliformis*) não foram considerados, pois não foram efetivamente publicados (OTT, 2009). Nesta revisão, reconhecemos quatro espécies já descritas (*S. delicatula*, *S. huillensis*, *S. suecica* e *S. kenneydyi*) com base nos dados moleculares, morfológicos e distribuição geográfica. Cinco espécies novas de *Sirodotia* foram propostas neste estudo: *S. amazonica*, *S. aquiloamericana*, *S. assamica*, *S. cryptica* e *S. delicatuliformis*. Portanto, nove espécies foram reconhecidas e mostraram-se nitidamente separadas por evidências moleculares com clados ou grupos bem definidos nas análises moleculares, caracteres morfológicos distintivos e padrão evidente de distribuição geográfica.

Tabela 4. Relação das espécies *Sirodotia* anteriormente consideradas taxonomicamente aceitas e as reconhecidas neste estudo. Os nomes em negrito indicam as nove espécies reconhecidas neste estudo.

Espécies anteriormente aceitas	**Espécies reconhecidas neste estudo**
S. acuminata	Sinonimizada com *S. suecica*
	***S. amazonica* (espécie nova)**
	***S. aquiloamericana* (espécie nova)**
S. assamica	***S. assamica***
S. ateleia	Sinonimizada com *S. delicatula*
S. cirrhosa	Duvidosa
	***S. cryptica* (espécie nova)**
S. delicatula	***S. delicatula***
	***S. delicatuliformis* (espécie nova)**
S. fennica	Sinonimizada com *S. suecica*
S. gardneri	Duvidosa
S. goebelii	Sinonimizada com *S. suecica*
S. huangshanensis	Duvidosa
S. huillensis	***S. huillensis***
S. kennedyi	***S. kennedyi***
S. segawae	Sinonimizada com *S. suecica*
S. sinica	Sinonimizada com *S. suecica*
S. suecica	***S. suecica***
S. tenuissima	Sinonimizada com *S. suecica*
S. yutakae	Sinonimizada com *S. suecica*

Fonte: Produção do próprio autor.

A descoberta de espécies novas dentro deste grupo confirma a segunda hipótese deste trabalho, visto que foram reconhecidas a existência de três espécies crípticas do Brasil (*S. amazonica, S. cryptica* e *S. delicatuliformis*) e uma espécie nova (*S. aquiloamericana*) na América do Norte, descrita anteriormente como *S. huillensis*, a qual apresentou valores de divergência altos (2,7-2,8% para *rbc*L e 6,8% para COI-5P), com relação ao espécime de *S. huillensis* da África, o suficiente para distingui-la como uma nova espécie.

Os caracteres morfológicos diagnósticos utilizados para distinguir o gênero (base carpogonial assimétrica e carposporófito difuso composto de filamentos prostrados produzindo ramos eretos determinados com carposporângios) foram confirmados como bons critérios. Alguns caracteres morfológicos anteriormente usados para distinguir as espécies do gênero (posição do carpogônio que produz os filamentos gonimoblásticos e dimensões dos carposporângios), foram corroborados pelos dados moleculares. Por outro lado, alguns caracteres morfológicos utilizados anteriormente

para distinguir espécies do gênero (espermatângios em ramos especializados, origem do ramo carpogonial, o comprimento do carpogônio, carpogônio curvado ou deflexo, presença de monosporângios nos fascículos secundários e sistema de cruzamento) foram aqui considerados possivelmente mal interpretados ou taxonomicamente não informativos e/ou apresentaram altas variações e sobreposições entre as espécies do gênero. Assim, os seguintes caracteres morfológicos foram considerados diagnósticos: número de células dos fascículos primários, arranjo dos espermatângios, posição do carpogônio que produz os filamentos gonimoblásticos e dimensões dos carposporângios. Considerando que alguns caracteres foram corroborados, mas outros foram considerados sem valor taxonômico ou mal interpretados, a terceira hipótese foi confirmada parcialmente.

Além dos dados moleculares e caracteres morfológicos, algumas espécies de *Sirodotia* apresentaram nítida repartição geográfica, que serviu como critério adicional para reconhecimento dessas espécies: *S. delicatuliformis* ocorrência no Brasil e Costa Rica, *S. amazonica* (regiões centro-oeste e norte do Brasil) e *S. cryptica* (centro-oeste do Brasil), *S. assamica* do nordeste da Índia, *S. aquiloamericana* do sul da América do Norte, *S. huillensis* do sul e sudeste da África, *S. kennedyi* do centro-sul da África. Estes dados evidenciaram a importância do padrão biogeográfico das espécies e confirmaram nossa quarta hipótese.

Encontramos níveis adequados de divergência entre sequências para separação dos grupos, com altos valores entre os grupos e mais baixos dentro dos grupos: 1,6-7% para *rbc*L e 4,2-10,5% para COI-5P, e 0-2,4% para *rbc*L e 0-3.8% para COI-5P, respectivamente. Esses valores encontram-se dentro dos limites de variação intra- e interespecífica reportados para espécies de outros gêneros da ordem Batrachospermales. O marcador LSU “barcode” não demonstrou ser um marcador apropriado por ser menos variável e, portanto, com menor poder de resolução para distinguir espécies, conforme indicado pelos menores valores de divergência entre os grupos formados e suporte relativamente mais baixo dos grupos nas árvores. Entretanto, ele tem sido usado como um bom marcador para inferir filogenias ou identificação de espécies em análises combinadas com outros marcadores, especialmente *rbc*L e UPA (Sherwood et. al., 2010). Alguns dados de sequências completas de LSU foram utilizados com sucesso em vários níveis taxonômicos incluindo circunscrição de gêneros e espécies para estabelecer novas ordens de algas vermelhas (por exemplo Harper & Saunders 2001; Huisman *et al*., 2004; Tronchin *et*

al., 2004). No trabalho de Harper & Saunders (2001), os dados para o marcador LSU para seis ordens de Florideophyceae, apresentou mesma topologia e maior valor de boostrap quando comparado com o SSU. No entanto, para sequências de algas vermelhas do grupo de Florideophyceae no trabalho de Sherwood *et al*. (2010), apesar do marcador LSU ser bem conservado, a análise concatenada de sequências de LSU com UPA e COI-5P gerou árvore de NJ com maior suporte nos níveis família e ordem. Dessa forma, o uso do marcador LSU neste trabalho serviu mais especificamente para compor a análise concatenada com os outros dois marcadores permitindo maior suporte para os clados terminais nas análises.

REFERÊNCIAS

AGOSTINHO, D. C; NECCHI, O. Jr. Systematics of the section *Virescentia* of the genus *Batrachospermum* (Batrachospermales, Rhodophyta) in Brazil. **Phycologia,** v.53, n. 5, 2014.

ENTWISLE, T. J.; VIS, M. L.; CHIASSON, W. B.; NECCHI, O. J.; SHERWOOD, A. R. Systematics of the Batrachospermales (Rhodophyta) - a synthesis. **Journal of Phycology,** v. 45, p. 704-715, 2009.

GUIRY, M. D.; GUIRY, G. M. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org (accessed 18 July 2020).

HANYUDA, T.; SUZAWA, Y.; SUZAWA, T.; ARAI, S.; SATO, H.; UEDA, K. Biogeography and taxonomy of *Batrachospermum helminthosum* (Batrachospermales, Rhodophyta) in Japan inferred from *rbc*L gene sequences. **Journal of Phycology,** v. 40, p. 581–8, 2004.

HARPER, J. T. & SAUNDERS, G. W. The application of sequences of the ribosomal cistron to the systematics and classification of the florideophyte red algae (Florideophyceae, Rhodophyta). **Cahiers Biologie Marine,** v. 42, p. 25 – 28, 2001.

HOUSE, D. L.; SHERWOOD, A. R.; VIS, M. L. Comparison of three organelle markers for phylogeographic inference in Batrachospermum helminthosum (Batrachospermales, Rhodophyta) from North America. **Phycological Research,** v. 56, n. 2, p. 69-75, 2008.

HOUSE, D. L.; VANDENBROEK, A. M.; VIS, M. L. Intraspecific genetic variation of Batrachospermum gelatinosum (Batrachospermales, Rhodophyta) in eastern North America. **Phycologia,** v. 49, n. 5, p. 501-507, 2010.

HUISMAN, J. M.; ABBOTT, L. A. & SHERWOOD, A. R. Large subunit rDNA gene sequences and reproductive morphology reveal Stenopeltis to be a member of the Liagoraceae (Nemaliales, Rhodophyta), with a description of Akalaphycus gen. nov. **European Journal of Phycology,** v. 9, p. 257-272, 2004.

LEONARDO DA VINCI. Pensador: colecione e compartilhe frases, poemas, mensagens e textos. Disponível em: https://www.pensador.com/autor/leonardo_da_vinci/. Acesso em: 20.03.2020.

NECCHI, O. JR., VIS, M.L. & OLIVEIRA, M.C. Phylogenetic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North and South America. **Cryptogamie, Algologie,** v. 27, p. 117-127, 2007.

OTT, F. D. *Handbook of the taxonomic names associated with the non-marine Rhodophycophyta*. J. Cramer, Stuttgart, 969 pp, 2009.

PAIANO, M. O.; NECCHI, O. JR. Phylogeography of the freshwater red alga *Sirodotia* (Batrachospermales, Rhodophyta) in Brazil. **Phycological Research**, v. 61, p. 249-255, 2013.

ROSSIGNOLO, N. L. & NECCHI, O. Jr. Revision of section *Setacea* of the genus Batrachospermum (Batrachospermales, Rhodophyta) with emphasis on specimens from Brazil. **Journal of Phycology,** v. 55 (4), p. 337-346, 2016.

SHERWOOD, A.R.; SAUVAGE, T.; KURIHARA, A.; CONKLIN, K.Y.; PRESTING G.G. A comparative analysis of COI, LSU and UPA marker data for the Hawaiian florideophyte Rhodophyta: implications for DNA barcoding of red algae. **Cryptogamie Algologie***,* v. 31, p. 451-465, 2010.

TRONCHIN, E. M.; DE CLERCK, O.; FRESHWATER, D. W.; BOLTON. J. J. & ANDERSON, R. J. Ptilophora leliaertii and Ptilophora coppejansii, two new species of Gelidiales (Rhodophyta) from South Africa. **European Journal of Phycology**, v. 39, p. 395-410, 2004.

VIS, M. L.; CAMERON HODGE, J.; NECCHI, O. J. Phylogeography of *Batrachospermum macrosporum* (Batrachospermales, Rhodophyta) from North and South America. **Journal of Phycology,** v. 44, n. 4, p. 882-888, 2008.

VIS, M.L. & ENTWISLE, T.J. Insights into the phylogeny of the Batrachospermales (Rhodophyta) from *rbc*L sequence data of Australian taxa. **J. Phycol**. 36: 1175-1182, 2000.

VIS, M. L.; FENG, J.; CHIASSON, W. B.; XIE, S.-L.; STANCHEVA, R.; ENTWISLE, T. J.; CHOU, J.-Y.; WANG, W.-L. Investigation of the molecular and morphological variability in *Batrachospermum arcuatum* (Batrachospermales, Rhodophyta) from geographically distant locations. **Phycologia,** v. 49, n. 6, p. 545-553, 2010.

VIS, M. L.; MILLER, E. J.; HALL, M. M. Biogeographical analyses of *Batrachospermum helminthosum* (Batrachospermales, Rhodophyta) in North America using molecular and morphological data. **Phycologia**, p. 40: 2–9, 2001.

VIS, M. L.; NECCHI, O. J.; CHIASSON, W. B.; ENTWISLE, T. J. Molecular phylogeny of the genus *Kumanoa* (Batrachospermales, Rhodophyta). **J. Phycol.,** v. 48, p. 750-758, 2012.

VIS, M.L., SAUNDERS, G.W., SHEATH, R.G., DUNSE, K.; ENTWISLE, T.J. Phylogeny of the Batrachospermales (Rhodophyta) inferred from *rbc*L and 18S ribosomal DNA gene sequences. **J. Phycol.** v. 34, p. 341-350, 1998.

VIS, M.L. & SHEATH, R.G. A molecular investigation of the systematic relationship of *Sirodotia* species (Batrachospermales, Rhodophyta) in North America. **Phycologia**. v. 38, p. 261-266, 1999.