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

**Natalia L. ROSSIGNOLO**
São Paulo State University, Zoology and Botany Department, Cristóvão Colombo, 2265, 15054-000 São José Rio Preto, São Paulo (Brazil)

**Morgan L. VIS**
Ohio University, Department of Environmental and Plant Biology, OH (United States)

**Monica O. PAIANO**
University of Hawaï, School of Life Sciences, Honolulu, HI (United States)

**Pertti ELORANTA**
Sinkilätie 13, Jyväskylä FI-40530 (Finland)

**John A. WEST**
School of Biosciences 2, University of Melbourne, Parkville VIC 3010 (Australia)

**E. K. GANESAN**
Oceanographic Institute, Universidad de Oriente, Cumaná (Venezuela)

**Farishta YASMIN**
Nowgong College, Botany Department, Nagaon, 782001, Assam (India)

**Phaik-Eem LIM**
Institute of Ocean and Earth Sciences (IOES), University of Malaya (Malaysia)

**Orlando Jr NECCHI**
São Paulo State University, Zoology and Botany Department, Cristóvão Colombo, 2265, 15054-000 São José Rio Preto, São Paulo (Brazil)
o.necchi@unesp.br (corresponding author)

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## ABSTRACT
Most genera of the freshwater red algal order Batrachospermales have been systematically revised using molecular and morphological data, but *Sirodotia* Kylin remains to be thoroughly reviewed. In this investigation, DNA sequence data for the *rbc*L, COI-5P and LSU markers of specimens collected worldwide were combined with morphological observations to assess their specific diversity, infer their relationships and evaluate the morphological characters relevant for species identification. Phylogenetic analyses showed the genus to be a monophyletic lineage with high support. Inter- and intra-specific 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. LSU sequences revealed

lower interspecific divergence values than the COI-5P sequences (0.7-3.3%) indicating less 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* Necchi, Rossignolo, Yasmin, J.A.West & Ganesan, *S. delicatula* Skuja, *S. huillensis* (Welwitsch ex West & GSWest) Skuja, *S. kennedyi* A.L.Szinte, J.C.Taylor & M.L.Vis and *S. suecica* Kylin) and four new species are proposed (*S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. aquiloamericana* Necchi, N.L.Rossignolo & M.L.Vis, sp. nov., *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.). Diagnostic characters for the genus are confirmed to be carpogonia asymmetric 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, *S. sinica, S. segawae and S. yutakae* are proposed as synonyms of *S. suecica* and *S. ateleia* Skuja of *S. delicatula*. The status of three species (*S. cirrhosa* Skuja ex M.S.Balakr. & B.B.Chaugule, *S. gardneri* Skuja ex Flint and *S. huangshanensis* Z.X.Shi & S.L.Xie) could not be confirmed due to lack of type specimens and published information on informative diagnostic characters.

**KEY WORDS**
Batrachospermales,
COI-5P,
freshwater red algae,
biogeography,
LSU,
molecular systematics,
*rbc*L,
*Sirodotia*

**RÉSUMÉ**
*Révision du genre* Sirodotia *Kylin (Batrachospermales, Rhodophyta) avec la description de quatre nouvelles espèces.*
Parmi les algues rouges d'eau douce de l'ordre des Batrachospermales, la délimitation des genres a été largement révisée à l'aide de données moléculaires et morphologiques, mais les *Sirodotia* Kylin doivent encore faire l'objet d'un examen en profondeur. Dans cette étude, les données de séquences d'ADN pour les marqueurs *rbc*L, COI-5P et LSU provenant d'échantillons prélevés dans le monde entier ont été combinées avec des observations morphologiques. Les analyses phylogénétiques ont montré que le genre formait un groupe monophylétique avec un soutien élevé. Les valeurs de divergence interspécifique et intra-spécifique étaient bien définies avec des variations interspécifiques plus élevées (2,1-7% et 4,4-10,5%) et intraspécifiques plus faibles (0-2,4% et 0-3,8%) pour les séquences *rbc*L et COI-5P, respectivement. Les séquences LSU ont révélé des valeurs de divergence interspécifique plus faibles que les séquences COI-5P (0,7-3,3%) indiquant une résolution inférieure en tant que marqueur de code à barres. Neuf espèces sont reconnues sur la base des données de séquence d'ADN, des caractères morphologiques et de répartition géographique. Cinq espèces ont été précédemment décrites (*S. assamica* Necchi, Rossignolo, Yasmin, J.A.West & Ganesan, *S. delicatula* Skuja, *S. huillensis* (Welwitsch ex West & G.S.West) Skuja, *S. kennedyi* A.L.Szinte, J.C.Taylor & M.L.Vis et *S. suecica* Kylin) et quatre nouvelles espèces sont proposées (*S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. aquiloamericana* Necchi, N.L.Rossignolo & M.L.Vis, sp. nov., *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. et *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.). Il est confirmé que les caractères diagnostiques du genre sont des carpogones asymétriques avec une protubérance basale et des carposporophytes diffus avec des filaments prostrés indéterminés produisant des branches dressées déterminées se terminant par des carposporanges. Les caractères morphologiques suivants ont été utilisés pour distinguer les espèces : nombre de cellules du fascicule primaire, disposition spermatangiale, origine du filament gonimoblastique et taille des carposporanges. Sur la base de la morphologie, trois espèces sont proposées comme synonymes de *S. suecica* (*S. sinica* CC Jao, *S. segawae* Kumano et *S. yutakae* Kumano) et *S. delicatula* (*S. ateleia*). Le statut de trois espèces (*S. cirrhosa* Skuja ex M.S.Balakr. & B.B.Chaugule, *S. gardneri* Skuja ex Flint and *S. huangshanensis* Z.X.Shi & S.L.Xie) n'a pas pu être confirmé en raison du manque de spécimens types ou d'informations publiées sur les caractères diagnostiques.

**MOTS CLÉS**
Batrachospermales,
COI-5P,
algue rouge d'eau douce,
biogéographie,
LSU,
systématique moléculaire,
*rbc*L,
*Sirodotia.*

## INTRODUCTION

Over the past two decades, the systematics of the freshwater red algal order Batrachospermales has been extensively studied using molecular and morphological data (e.g., Vis *et al.* 1998, Entwisle *et al.* 2009). Recent revisional studies of sections within *Batrachospermum* have rectified the paraphyly of this genus in relation to other genera of the order and resulted in most sections being raised to generic rank (Salomaki *et al.* 2014; Rossignolo & Necchi 2016; Necchi *et al.* 2018, 2019a, b; Vis *et al.* 2020). Using a combination of morphological observations and DNA sequence data, numerous new genera have been established within the order as a whole: *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; 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 these new genera, *Nothocladus*, *Sirodotia* and *Tuomeya* had long been recognized as distinct based on morphological characters (Kaczmarczyk *et al.* 1992; Necchi *et al.* 1993; Sheath *et al.* 1996). The genus *Nothocladus* was recently investigated and recircumscribed 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 from other genera in the Batrachospermales 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), in his summary of freshwater red algal knowledge at the time, recognized 19 species of *Sirodotia*. Kumano (2002), in his world monograph of freshwater red algae summarizing more recent research, lowered the number to eight species: *S. yutakae* Kumano, *S. segawae* Kumano, *S. sinica* Jao, *S. goebelii* Entwisle & Foard, *S. suecica* Kylin, *S. gardneri* Skuja ex Flint, *S. huillensis* (Welwitsch ex West & G.S.West) Skuja and *S. delicatula* Skuja. Five of these species were known only from the type locality or a few locations. Other general studies on freshwater Rhodophyta that included species of *Sirodotia* were 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 to date, and in that study he accepted only six species. However, there are 14 validly published and taxonomically accepted species (Ott 2009; Guiry & Guiry 2020; Rossignolo *et al.* 2020; Szinte *et al.* 2020; Fisher *et al.* 2020): *S. assamica* Necchi, Rossignolo, Yasmin, J.A.West & Ganesan, *S. cirrhosa* Skuja ex M.S.Balakr. & B.B.Chaugule, *S. delicatula* Skuja, *S. gardneri* Skuja ex Flint, *S. huangshanensis* Z.X.Shi & S.L.Xie, *S. huillensis* (Welwitsch ex West & G.S.West) Skuja, *S. yengarii* M.Baluswami & M.Babu, *S. kennedyi* A.L.Szinte, J.C.Taylor & M.L.Vis, *S. masoalensis* E.Fischer, D.Killmann & D.Quandt, *S. polygama* Skuja ex Flint, *S. segawae* Kumano, *S. sinica* C.C.Jao, *S. suecica* Kylin and *S. yutakae* Kumano.

The most commonly used diagnostic characters for species of *Sirodotia* have been: the shape and form of the gametophyte, presence or absence of spermatangia on specialized branches, origin of the carpogonial branch (protuberant or non-protuberant side of the carpogonium base), and size and shape of the carpogonium and carposporangia (Necchi *et al.* 1993, 2007; Kumano 2002). However, some overlap in morphometric characters among species has been noted. Necchi (1991) reported that the length of the carpogonial branch and the shape and size of the carpogonia showed wide and continuous variation in many populations of *S. delicatula* from Brazil, suggesting that these characters alone should not be used for the delimitation of this and other species in the genus *Sirodotia*. Another character sometimes used for identification has been 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) mode of reproduction is not considered useful in distinguishing species of Batrachospermales given that polyecious populations (with both monoecious and dioecious individuals) are frequent in the family. Thus, further investigation of diagnostic characters for all species is required.

Species of the genus have been reported from all continents, except Antarctica (Necchi *et al.* 1993, 2007; Entwisle & Foard 1999; Sheath & Sherwood 2002; Carmona *et al.* 2006; Eloranta & Kwandrans 2007; Eloranta *et al.* 2011), but the diversity of species is variable across the world. Necchi (1991) recognized only one species for Brazil (*S. delicatula*) with wide morphological variation and geographic distribution. Kumano (1982) accepted six species in Japan and Malaysia, including the description of two new species (*S. segawae* and *S. yutakae*). Necchi *et al.* (1993) analyzed 25 populations and ten type-specimens of *Sirodotia* in North America and recognized three species in this large region: *S. huillensis*, *S. suecica* and *S. tenuissima*.

A few species are considered to be cosmopolitan (Vis & Sheath 1999; Necchi *et al.* 2007; Lam *et al.* 2012; Johnston *et al.* 2014; Szinte *et al.* 2020), with *S. suecica* extending through temperate regions of North America and Europe as well as Australasia and Africa, and *S. delicatula* and *S. huillensis* in tropical and subtropical regions of the Americas and Asia. Molecular studies have all shown the genus to be monophyletic (Vis *et al.* 1998; Vis & Sheath 1999; Necchi *et al.* 2007; Entwisle *et al.* 2009) and some species to be superfluous or paraphyletic. Vis & Sheath (1999) analyzed North American collections identified as *S. suecica*, *S. tenuissima* and *S. huillensis* and concluded that *S. huillensis* is a distinct species, but *S. tenuissima* and *S. suecica* were synonymous based on molecular sequence similarity of specimens with the morphology of those two species. More recently, sequence data of *S. delicatula* from Brazil revealed it to be distinct in both morphology and molecular data from *S. huillensis* and *S. suecica* (Necchi *et al.* 2007). Lam *et al.* (2012) suggested that a specimen from South Africa, *Sirodotia* aff. *huillensis*, based on sequence data could be described as a separate species from *S. suecica*, *S. delicatula* and *S. huillensis*. In addition, molecular studies on *Sirodotia delicatula* populations from Brazil (Paiano & Necchi 2013) indicated the existence of cryptic *Sirodotia* species. Johnston *et al.* (2014) showed that

*S. delicatula* from Malaysia and Brazil are not genetically similar and should be considered separate taxa. Given the closer proximity to the type locality (Indonesia) of *S. delicatula*, they proposed that the Malaysian specimen be retained in *S. delicatula*, while the Brazilian species be considered a distinct (cryptic) species yet to be described.

With the access now to additional DNA sequence data from a wide range of taxa and geographical range, it is possible to evaluate the species-level taxonomy and phylogenetic relationships of the entire genus *Sirodotia*. This also provides an opportunity to reappraise the morphological characters used for species delimitation. The goals of this study were to: 1) infer the phylogenetic relationships among species of the genus *Sirodotia* worldwide, based on 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); and 2) compare these results to a morphological analysis of the material from which sequences were derived and evaluate the characters used to circumscribe species.

## MATERIAL AND METHODS

### Morphological analyses

The type specimens of all species assigned to the genus *Sirodotia* were requested for morphological analysis. Those specimens obtained were (herbarium acronyms according to Thiers 2021):

1. *Sirodotia angolensis* (West & G.S.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 & G.S.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*, *S. huangshanensis*, *S. iyengarii* and *S. sinica* were requested, but not available for examination, so information from the protologues were used for morphological comparison. The type specimens of *S. gardneri* and *S. delicatula* could not be analyzed due to limited material. However, the morphometric data from the type specimens were available in Necchi *et al.* (1993).

In addition to types, specimens identified as *Sirodotia* were examined and sequenced from numerous regions of the world, including Africa, Asia, Australasia, Europe, North America and South America (Appendix 1). There was insufficient material of a few samples to allow morphological analysis (CR1, MEX1, FI3, FI4, FI5 and AU1, see Appendix 1), but where possible specimens were examined for all 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, 2007; Vis & Sheath 1999; Kumano 2002; Lam *et al.* 2012). For morphometric analyses, where possible 15 measurements or observations were taken for each character per sample, consistent with the protocol used in previous studies (Necchi 1990, Agostinho & Necchi 2014, Rossignolo & Necchi 2016). For some samples, 15 measurements were not possible, especially for reproductive structures (e.g., carpogonium and carposporangium dimensions). For morphological observations, the same procedures were applied as described in those studies.

### 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 TopTaqTM Master Mix Kit (Qiagen GmbH, Hilden, Germany) and GoTaq G2 Hot Star Polymerase (Promega, Madison, United States), 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 (1282 bp) and nuclear LSU barcode region (*c.* 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 & Sheath (1999). The LSU barcode region was amplified using the primers T16N and T24U (*c.* 600 bp) and thermocycler settings provided in Saunders & Moore (2013). For purification of PCR products NucleoSpin Gel and PCR Clean-Up (Macherey-Nagel, Bethlehem, PA, United States) was used according to manufacturer's protocols. Amplification of the LSU barcode region occasionally resulted in multiple bands, requiring an additional purification step of the excised desired band from the electrophoresis gel.

Purified PCR products were subjected to sequencing reactions using the ABI PRISM Big Dye Terminator kit. 3.1 (Applied Biosystems, Foster City, CA, United States) 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.

For each gene region (*rbc*L, COI-5P and LSU), the new sequences (Appendix 1) and other available sequences of *Sirodotia* and Batrachospermales from GenBank database, National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov, Benson *et al.* 2013; Appendix 3) were aligned using the Geneious software version 7 (http: //www.geneious.com, Kearse *et al.* 2012). The LSU marker 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 three gene regions (*rbc*L, COI-5P and LSU), 35 with two gene regions (32 for *rbc*L and COI-5P; two for *rbc*L and LSU; one for COI-5P and LSU), 19 with one gene region (Appendices 1-3, 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. The COI-5P sequences were also analyzed for the existence of "barcode gaps", namely, difference between interspecific and intraspecific variation (Leliaert *et al.* 2014) using the single gene method of "Automatic Barcode Gap Discovery" (ABGD; Puillandre *et al.* 2012; http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html).

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 analyzelys 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 generations. 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).

## RESULTS

### Molecular analyses

Seven *rbc*L, 19 COI-5P and 26 LSU sequences were newly generated for this study (Appendix 1). To complete the data set, 14 *rbc*L, three COI-5P and two LSU sequences were obtained from Genbank (Appendices 2, 3). 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. 1; Appendix 7).

All phylogenetic analyses of the concatenated and single gene data showed similar results with high support values (bs and pp) 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 analysed, including the LSU sequence of the *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. from Brazil (BR21), for which it was not possible to obtain sequences for *rbc*L and COI-5P. The ML tree of concatenated data and NJ tree for COI-5P are shown (Figs 1, 2), whereas the ML tree for *rbc*L and NJ tree for LSU are presented as supplemental materials (Appendices 7, 9). The genus *Sirodotia* formed a well-supported lineage (>95 bs and >0.95 pp) in all analyses (Figs 1, 2; Appendices 7,-9), showing that the genus *Sirodotia* represents a monophyletic group within a large lineage of representative sequences for all genera of Batrachospermales (Fig. 1; Appendix 7). All lineages of genera had high support values, but relationships among them were mostly poorly supported.

Within the genus *Sirodotia*, there were two major lineages with high support values: one composed of *S. suecica* sequences from numerous regions of the world (Africa, North America, Europe and Australasia), the other with sequences of all other species, again from several continents (Africa, North America, South America and Asia) (Fig. 1). There were five lineages 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* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. aquiloamericana*, *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. As well, there were individual sequences that were more distantly related to these lineages and those were determined to be *S. delicatula, S. huillensis* and *S. kennedyi*. In the lineage of *S. suecica*, a sample from South Africa (JN408524) was on a longer branch with high support (>95 bs and >0.95 pp). The recently described species *S. masoalensis* differs in only 0.6% of *S. kennedyi* in *rbc*L sequences.

*Sirodotia kennedyi* (Zambia) was sister to a lineage from South America, *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., 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 lineages (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 COI-5P NJ tree (Fig. 2) with 19 new COI-5P sequences generated in this study combined with three GenBank *Sirodotia* sequences showed well-supported groups representing eight species. COI-5P sequence was not available for *S. kennedyi* but only for *S. masoalensis*, which was positioned on a long branch and distant (5.1-6.5%) from the closest species (*S. delicatuliformis* Necchi, N.L.Rossignolo &

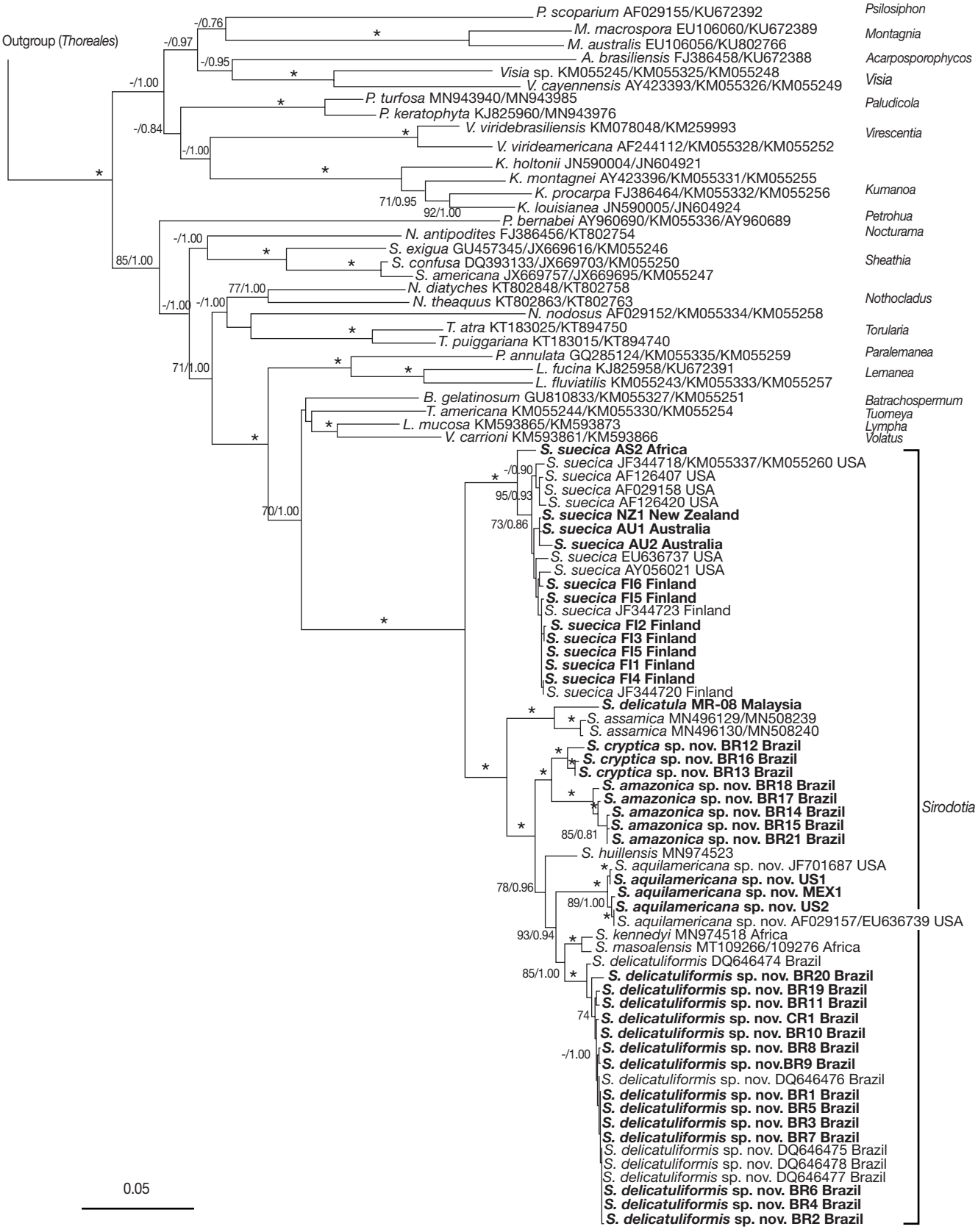



FIG. 1. — Phylogenetic tree (ML) showing the relationship of species within *Sirodotia* Kylin and genera of the Batrachospermales and Thoreales based on *rbc*L, COI-5P and LSU concatenated sequence data. * = bootstrap (bs)>95 and posterior probability (pp)>0.95 support; nodes without values indicate bs<70 and pp<0.80. Specimen code as in Appendix 1. Some sequences represent more than one collection with identical.

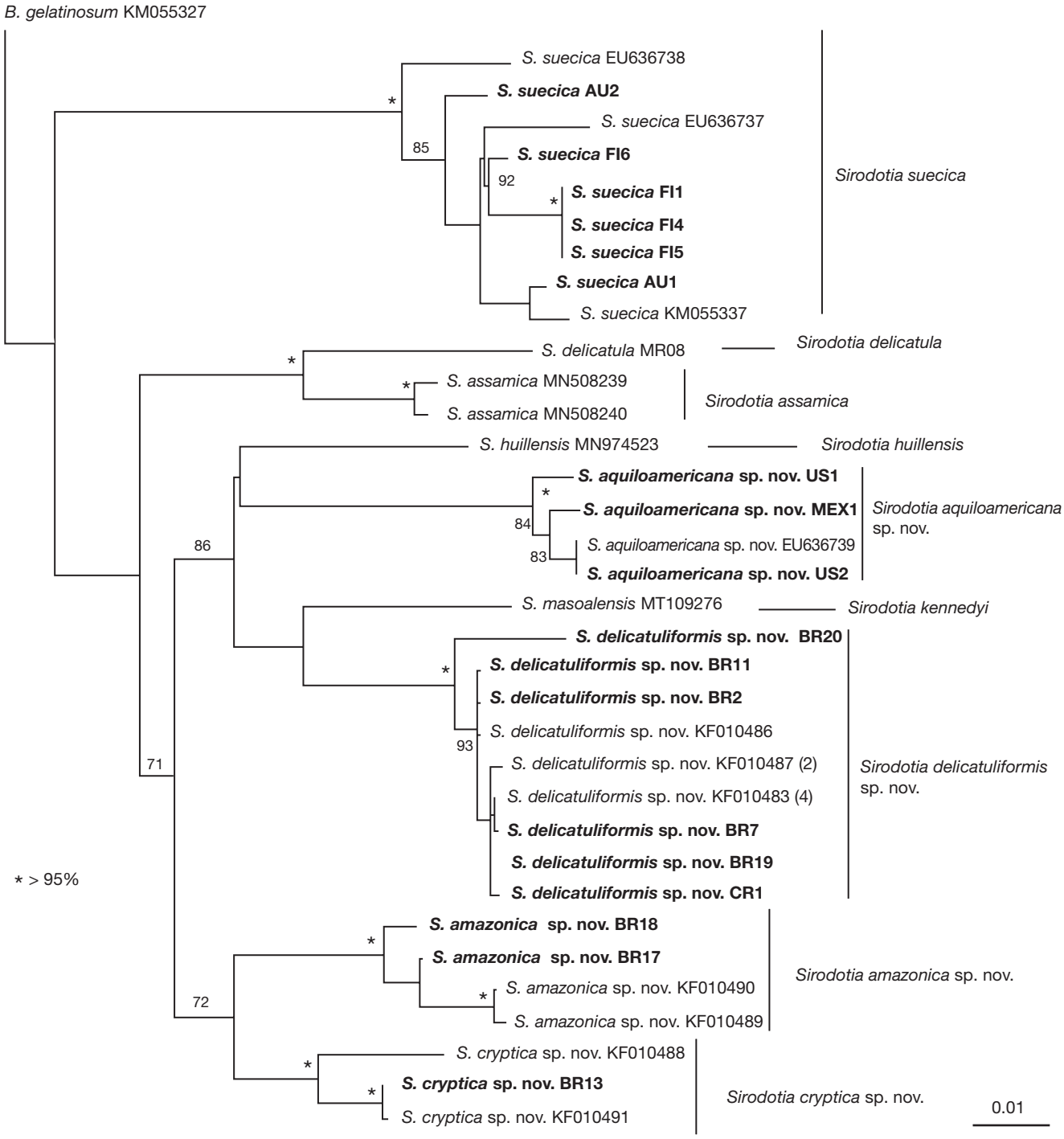



FIG. 2. — Neighbor Joining tree (NJ) showing the relationship of Sirodotia Kylin species based on COI-5P sequence data. * = bootstrap (bs)>95; nodes without values indicate <70 bs. Specimen code as in Appendix 1. Some sequences represent more than one collection with identical sequence, the number of additional sequences is shown in parentheses and this information is noted in Appendix 1. Scale represents substitutions per site.

M.O.Paiano, sp. nov.). 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* sp. nov., *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. The lineage 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 containing seven species exhibited high interspecific divergences (3-4.2%) and low divergence within groups (*c.* 2.5%). Species delineation analysis using

ABGD for the COI-5P barcode region (Appendix 9) with 38 sequences showed partitioning into eight groups, which were consistent with the concatenated data set and the single gene trees (Figs 1, 2, Appendices 7, 9).

The *rbc*L tree was mostly congruent with the concatenated tree and exhibited two major lineages, one with *S. suecica* and the other with all other species (Appendix 7). The one lineage containing the sequences of *S. suecica* showed a wide intraspecific variation (0.1-2.4%). The other large lineage had seven smaller lineages: 1) two species - *S. delicatula* from Malaysia (high support bs >95%, pp >0.90) and a recently described species (*S. assamica* from India, intraspecific divergence 0.4%); 2) a sequence previously referred as *S. aff. huillensis* from Africa (as argued later in this paper, considered to be 'true' *S. huillensis)* diverging from the closer species *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. in 2.1-3.2%; 3) samples of *S. cryptica* from Midwestern Brazil (states of Goiás and Mato Grosso,) with a variation of 0.2-0.8%; 4) four samples of *S. amazonica* from Brazil (states of Mato Grosso and Roraima, divergence of 0-0.3%); 5) samples of *Sirodotia* from North America previously referred as *S. huillensis* (divergence 0-0.5%), here proposed as a new species (*S. aquiloamericana*); 6) a recently described species from Africa (*S. kennedyi*, including *S. masoalensis* different in only 0.6%) that diverged from the neighboor species *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. by 1.6-1.9%; and 7) sequences from southeastern Brazil and a sample from Costa Rica (AF126410) referred to as *S. delicatula* (within lineage divergence 0-0.7%), and here interpreted as a new species (*S. delicatuliformis* sp. nov.). In summary, the phylogenetic analysis of the *rbc*L marker showed nine species of *Sirodotia*, of which five already have valid names (*S. assamica*, *S. delicatula*, *S. huillensis*, *S. kennedyi* and *S. suecica*) and four require taxonomic recognition and are described here (*S. amazonica* sp. nov., *S. aquiloamericana*, *S. cryptica* sp. nov. and *S. delicatuliformis* sp. nov.).

The LSU NJ tree (Appendix 9) 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* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.*, S. assamica, S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.*, S. delicatula* and *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. 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* sp. nov.*, S. assamica*, *S. cryptica* sp. nov., *S. delicatula*, *S. delicatuliformis* sp. nov., and *S. suecica*.

### Morphological analyses

Among the 40 samples of *Sirodotia* available for molecular data (Appendix 1), 25 had sufficient specimens for morphological analysis. Of those 25 samples, all shared the characterisitic features of the genus (Figs 3-6, Appendices 4-6). Two characters common to all specimens were the obconical or pear-shaped whorls (Figs 3A, F, K; 4F; 5A, F; 6A, J) 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. Characters useful for distinguishing species consistent with the molecular data include: 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.

### Taxonomic treatment

## ***Sirodotia*** Kylin

*Nova acta Regiae Societatis Scientiarum Upsaliensis*, ser. IV, 3: 38 (Kylin 1912).

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

Type species. — *Sirodotia suecica* Kylin, *Nova acta Regiae Societatis Scientiarum Upsaliensis* Ser. IV, 3: 38 (1912).

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

*Revised description*

Plants monoecious, dioecious or polyoecious, bluish green to yellowish green; branching irregular; whorls well-developed or reduced, contiguous or separated, obconical 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

KEY TO THE SPECIES OF THE GENUS *SIRODOTIA* KYLIN

1. Carposporangia small, 5-10 µm in length ..... 2
— Carposporangia large, 10-21 µm in length ..... 3

2. Primary fascicle 3-5 cells; erect gonimoblast filaments with 1 cell ..... *S. kennedyi* A.L.Szinte, J.C.Taylor & M.L.Vis
— Primary fascicle (5-)6-10 cells; erect gonimoblast filaments with 2-4 cells ..... *S. huillensis* (Welwitsch ex West & G.S.West) Skuja

3. Spermatangia arranged in clusters ..... *S. assamica* Necchi, N.L.Rossignolo, F.Yasmin, J.A.West & Ganesan
— Spermatangia isolated or in groups of 2-3 ..... 4

4. Gonimoblast initial developing from the non-protuberant side of the carpogonium ..... *S. suecica* Kylin
— Gonimoblast initial developing from the protuberant side of the carpogonium ..... 5

5. Known distribution restricted to Asia (Indonesia, Japan and Malaysia) ..... *S. delicatula* Skuja
— Known distribution in the Americas ..... 6

6. Carposporangia wide, 8-13 µm in diameter ..... *S. amazonica* sp. nov. and *S. cryptica* sp. nov
— Carposporangia narrow, 6-8.5 (-9.5) µm in diameter ..... 7

7. Known distribution in southern North America (Costa Rica) and South America (Brazil) ..... *S. delicatuliformis* sp. nov.
— Known distribution in arid regions of North America (United States, Mexico) ..... *S. aquiloamericana* sp. nov.

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 (obconical 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), *Paludicola* (Vis *et al.* 2020) and to varying degrees in other genera of Batrachospermales.

SPECIES DESCRIPTION

***Sirodotia amazonica***
Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.
(Fig. 3A-E)

TYPE. — O. Necchi Jr., 27.IX.2010, (holo-, SJRP[SJRP 31924]).

TYPE LOCALITY. — Brazil, Mato Grosso, River Rosana, Route BR-163, between Sinop and Sorriso; 11°57'26"S, 55°31'01"W.

ADDITIONAL SPECIMENS EXAMINED. — SJRP 32139, SJRP 32140, SJRP 32576, SJRP 32577, and SJRP 32578 (Appendix 1).

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, MW053464), *rbc*L (KC951866, KC951867, MW053480) and LSU (BR14, BR15, MW053499).

*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.

*Remarks*

The phylogeography study by Paiano & Necchi (2013) showed the existence of two cryptic species in Brazil. 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*

Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. is very similar to two species found in Brazil, *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., overlapping for most morphometric and morphological characters. However, *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. is distinguishable from *S. delicatuliformis* sp. nov. based on the wider carposporangia (8-13 versus 6-8.5(-9.5) µm in diameter).

### *Sirodotia aquiloamericana*
Necchi, N.L.Rossignolo & M.L.Vis, sp. nov.
(Fig. 3F-J)

TYPE. — T.A. Dempster, 24.IV.2011 (holo-, BHO[BHO-0437]).

TYPE LOCALITY. — United States, Arizona, outlet canal of Montezuma; 34°38'57"N, 111°45'08"W.

ADDITIONAL SPECIMENS EXAMINED. — BHO A-0410, MEX1.

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 (MW053469, MW053470, EU636739) and *rbc*L (JN408523, JF344716, AF126414).

*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.

*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. Thus, the North American material represents a distinct species that is here described. This species is most closely comparable to *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. based on the narrow carposporangia, 6-8.5 (-9.5) µm in diameter. However, *S. aquiloamericana* differs from *S. delicatuliformis* sp. nov. 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).

### *Sirodotia assamica*
Necchi, N.L.Rossignolo, F.Yasmin, J.A.West & Ganesan
(Fig. 4A-E)

*Phytotaxa* 437: 125 (2020).

TYPE. — F. Yasmin, 25.II.2019 (holo-, SJRP[SJRP 32584]).

TYPE LOCALITY. — India, Assam, Nagaon District, Chapanalla; 26°19'13.7"N, 92°10'16.5"E.

ADDITIONAL SPECIMENS EXAMINED. — SJRP 32583, SJRP 32585 (Appendix 1).

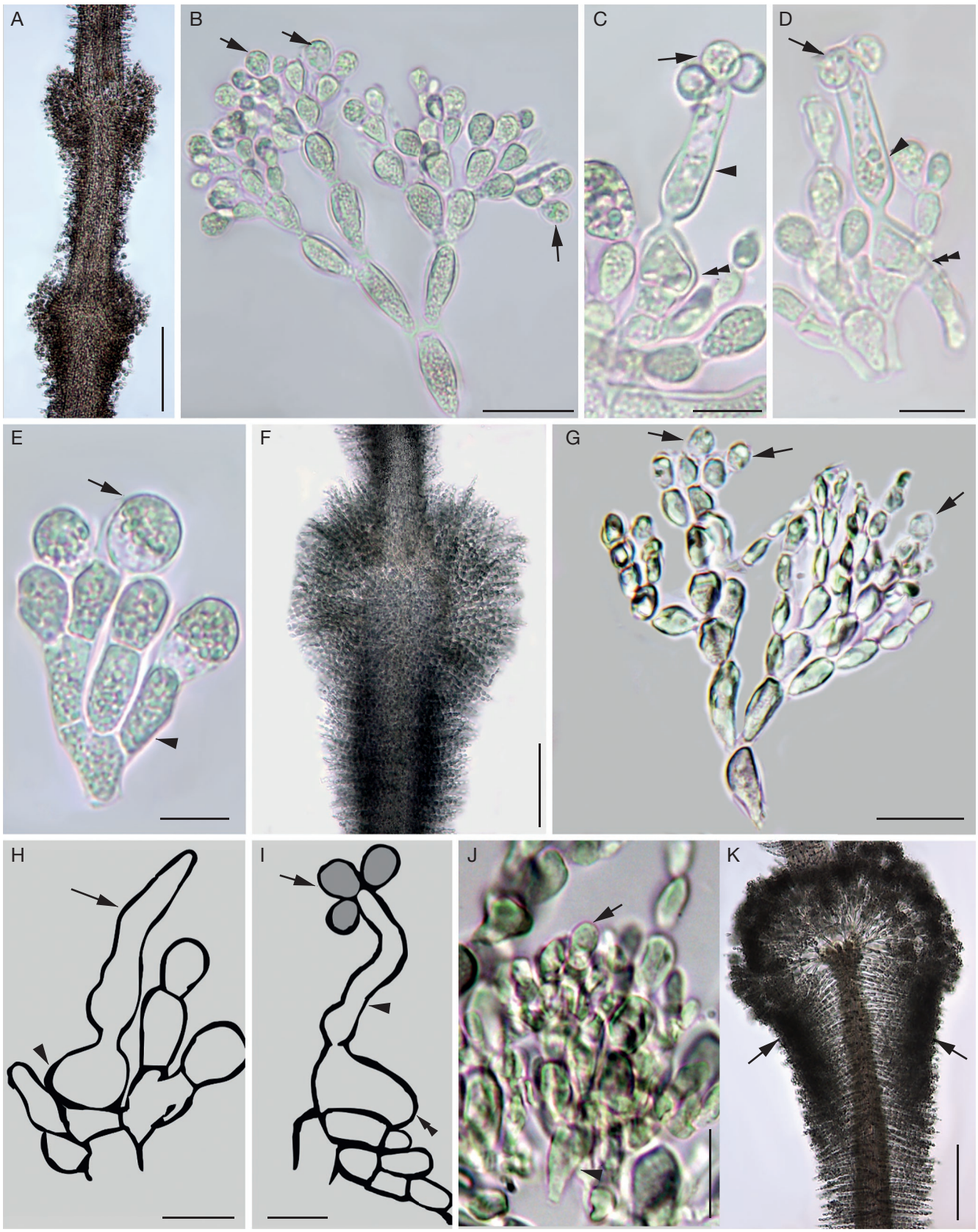
DISTRIBUTION. — Asia, India (northeastern).

REPRESENTATIVE DNA SEQUENCES. — COI-5P (MN508239, MN508240) and *rbc*L (MN496129, MN496130).

*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.

*Remarks*
A distinguishing feature of *Sirodotia assamica* 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 other vegetative and

FIG. 3. – Morphological features of *Sirodotia* species: **A**-**E**, *Sirodotia amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. (all figures taken from sample SJRP 31924 - holotype): **A**, pear-shaped whorls; **B**, primary fascicle with spermatangia (**arrow**); **C**, carpogonium with a mature trichogyne (**arrowhead**) showing the basal hemispherical protuberance (**double arrow**) and attached spermatia (**arrow**); **D**, fertilized carpogonium with a cylindrical trichogyne (arrowhead), attached spermatia (**arrow**) and gonimoblast initial developing from the protuberant side of the carpogonial base (**double arrow**); **E**, carposporangia (**arrow**) and erect (**arrowhead**) gonimoblast filaments. **F**-**J**. *Sirodotia aquiloamericana*: **F**, pear-shaped whorls; **G**, primary fascicle with spermatangia (**arrow**); **H**, carpo-




gonium with a mature trichogyne (**arrow**) showing the basal hemispherical protuberance (**arrowhead**); **I**, fertilized carpogonium with a cylindrical trichogyne (**arrowhead**), attached spermatia (**arrow**) and gonimoblast initial developing from the protuberant side of the carpogonial base (double **arrow**); **J**, carposporangia (**arrow**) and erect (**arrowhead**) gonimoblast filaments. **K**, *Sirodotia assamica* Necchi, N.L.Rossignolo, F.Yasmin, J.A.West & Ganesan (photo taken from sample SJRP 32583): pear-shaped whorls showing densely arranged spermatangia (**arrows**). Scale bars: A, F, K, 200 µm; B, G, J, 20 µm; C-E, H, I, 10 µm. H-I, redrawn from Necchi *et al*. (1993).

reproductive characteristics and its occurrence in or near India (Appendix 6). *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.

### *Sirodotia cryptica*
Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.
(Fig. 4F-J)

Type. — F.R. Borges, 15.IV.2014, (SJRP[SJRP 32575]).

Type locality. — Brazil, Goiás, Highway GO-070, between the municipalities of Jussara and Itaberaí; 16°00'32.7"S, 48°55'55.9"W.

Additional specimens examined. — SJRP 31923 and SJRP 31925 (Appendix 1).

Etymology. — The species epithet indicates that the alga is cryptic with another species described in this study (*S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.).

Distribution. — South America, Brazil (midwestern).

Representative DNA sequences. — COI-5P (KF010488, KF010491, MW053463,), *rbc*L (KC951865, KC951869, MW053479) and LSU (MW053494, MW053495, MW053498).

*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.

*Remarks*
This species is morphologically indistinguishable from *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. that also occurs in Brazil, but is genetically divergence and thus, it is here described as a new species.

### *Sirodotia delicatula* Skuja
(Fig. 5A-E)

*Archiv für Hydrobiologie Supplement* 15: 614 (Skuja 1938). — *Batrachospermum delicatulum* (Skuja) Necchi & Entwisle, *Phycologia* 29: 486 (1990).

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

Type. — D.L. Sunda-Expedition, 19.IX.1928 (lecto-, UPS[UPS A-003747]).

Type locality. — Indonesia, Java Island, Bogor, Tijiliwong; 6°35'21"S, 106°48'19"E.

Additional specimens examined. — BHO A-0984 (Appendix 1).

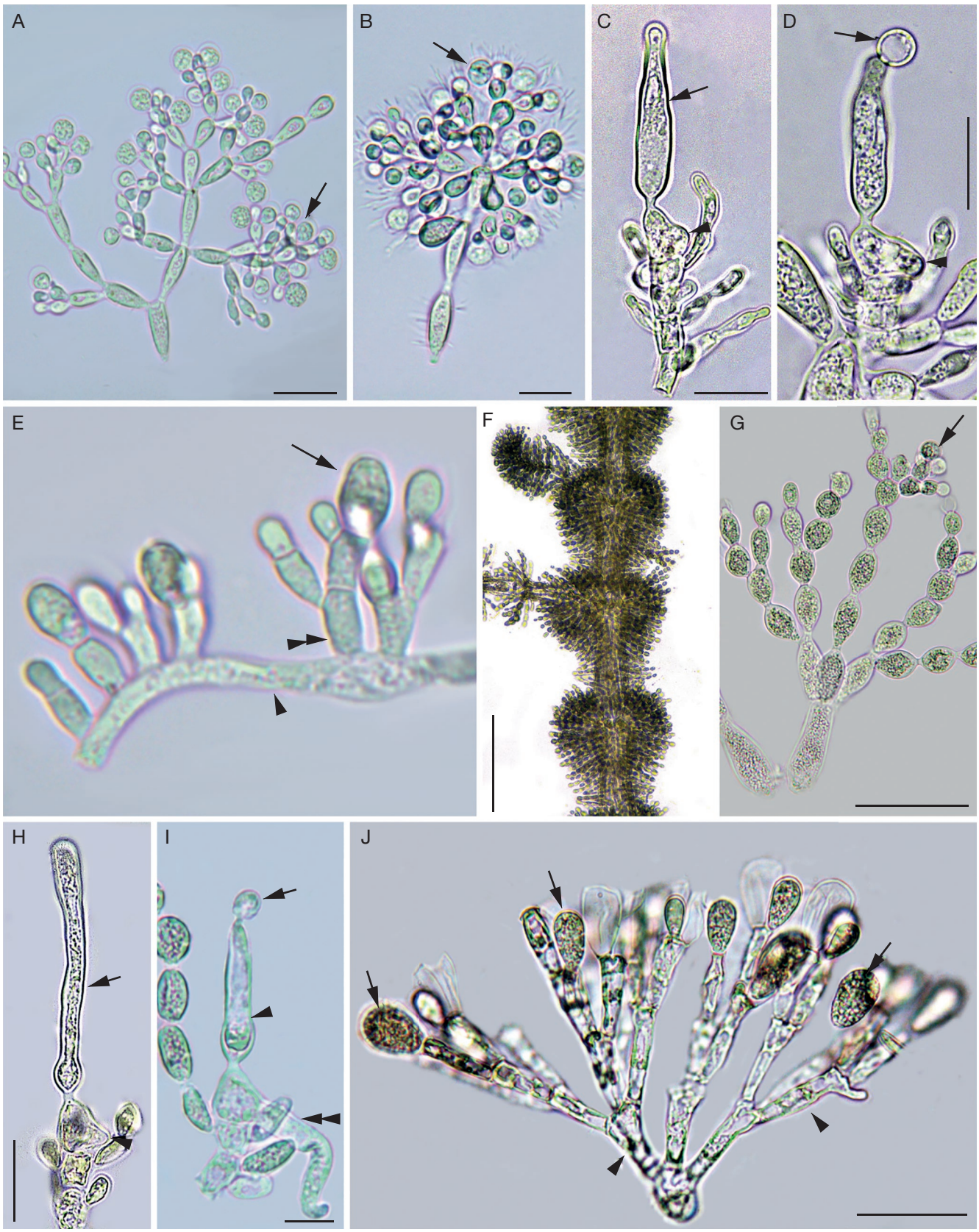
Distribution. — Asia: Indonesia, Japan and Malaysia.

Representative DNA sequences. — COI-5P (MW176122), *rbc*L (KF557560) and LSU (MW053507).

*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.

*Remarks*
This species is morphologically similar to four species of *Sirodotia* (*S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. aquiloamericana*). Like these species, it has 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 only in its 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

Fig. 4. – Morphological features of *Sirodotia* species. **A-E**, *Sirodotia assamica* Necchi, Rossignolo, Yasmin, J.A.West & Ganesan (photos taken from samples SJRP 32583, 32584): **A**, **B**; primary fascicles with spermatangia in clusters (**arrows**); **C**, a carpogonium with a mature trichogyne (arrow) showing the basal hemispherical protuberance (**arrowhead**); **D**, fertilized carpogonium with attached spermatium (**arrow**), gonimoblast initial (**arrowhead**) developing from the same side of the basal protuberance (**arrowhead**); **E**, carposporangia (**arrow**), prostrate (**arrowhead**) and erect (**double arrowhead**) gonimoblast filaments; **F-J**, *Sirodotia cryptica*




Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. (photos taken from sample SJRP 32575 - holotype): **F**, obconical whorls; **G**, primary fascicle with spermatangia (**arrow**); **H**, carpogonium with a mature cylindrical trichogyne (**arrow**) showing the basal hemispherical protuberance (**arrowhead**); **I**, fertilized 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); **J**, carposporangia (**arrows**) and erect gonimoblast filaments (**arrowheads**). Scale bars: A, B, G, 50 µm; C, D, H-J, 20 µm; E 10 µm; F, 200 µm. B-D, reproduced with permission from Rossignolo *et al.* (2020) (Magnolia Press).

South American ones based on the divergences in the DNA sequences 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 a synonym of *S. delicatula* by Umezaki (1960) and Necchi (1991) based on a combination of morphological 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* being synonymous 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, N.L.Rossignolo & M.O.Paiano, sp. nov.
(Fig. 5F-L)

Type. — O. Necchi Jr., 25.VI.2008, (holo-, SJRP[SJRP 31918].

Type locality. — Brazil, São Paulo State, Mirassol, São José dos Dourados River; 20°48'45"S, 49°34'29"W.

Distribution. — South America: Brazil (southeastern) and Central America: Costa Rica.

Representative DNA sequences. — COI-5P (KF010483, KF010486 e KF010487), *rbc*L (KC951857, KC951858, KC951863) and LSU (MW053486, MW053487, MW053491).

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 (Appendix 1).

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

*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.

*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 *S. delicatula* from Malaysia. Thus, it represents a distinct species that is here described. The species is highly divergent in sequence from *S. delicatula.* 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 lineage with three other species (*S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. aquiloamericana*) from North and South America. The morphology among these species is similar, but *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. 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
(Fig. 6A-E)

*Archiv für Protistenkunde* 74: 304 (Skuja 1931). — *Batrachospermum huillense* Welwitsch ex West & G.S.West, *Journal of Botany* 35: 3 (West & West 1897).

Type. — F. M. J. Welwitsch, V.1860 (holo-, BM[BM 001043858]; iso-, LISU).

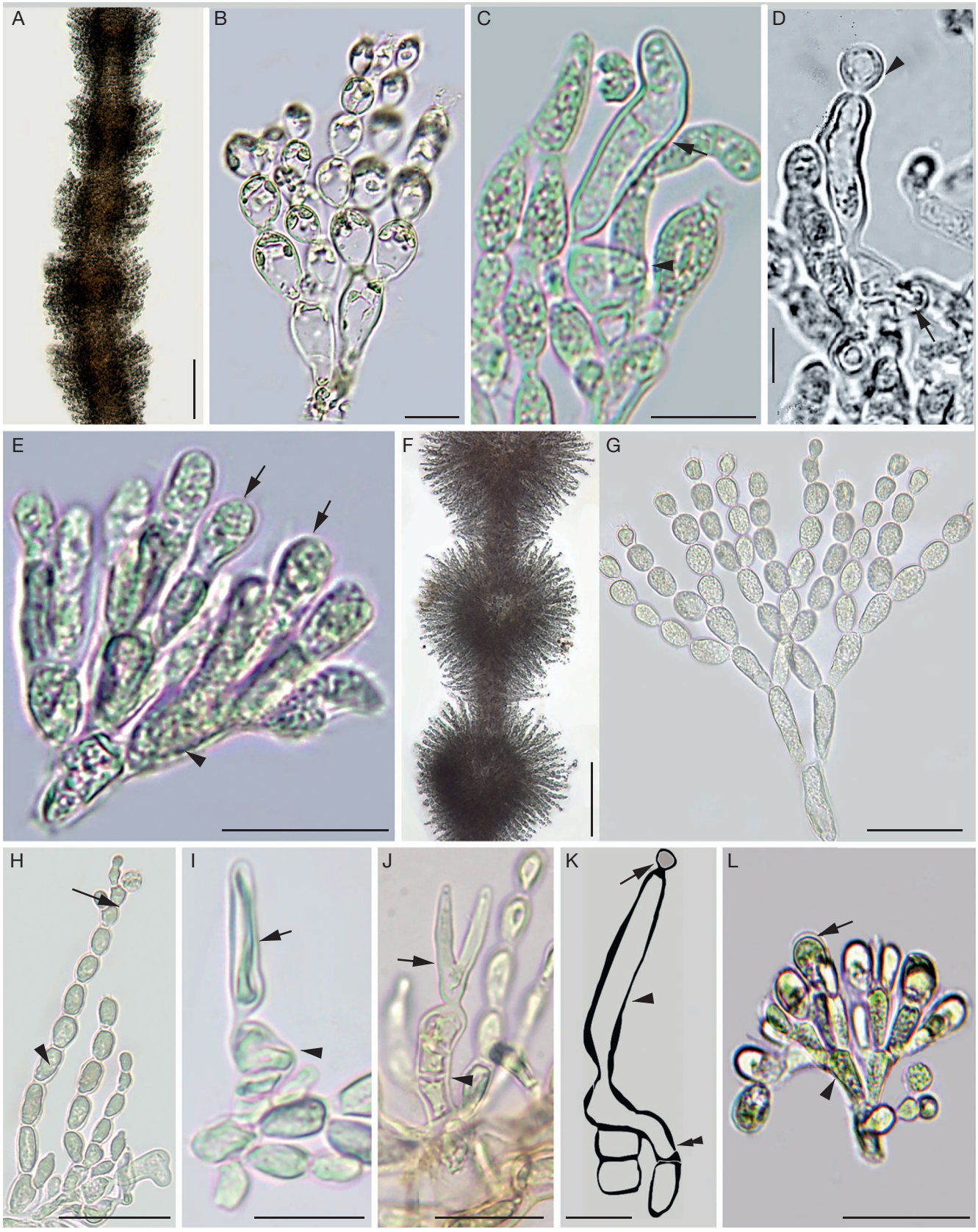
Type locality. — Africa, Angola, Huila, Lopollo, 14°47'51"S, 14°40'03"E.

Additional specimen examined. — BHO A-1447 (Appendix 1).

Distribution. — Africa: Angola, Madagascar, Reunion, South Africa.

Representative DNA sequences. — COI-5P (MN974523) and *rbc*L (JF344717).

Fig. 5. – Morphological features of *Sirodotia* species: **A-E**, *Sirodotia delicatula* Skuja (photos taken from sample BHO A-0984): **A**, obconical whorls; **B**, primary fascicle; **C**, carpogonium with a mature trichogyne (**arrow**) showing the basal hemispherical protuberance (arrowhead); **D**, fertilized carpogonium with a sessile cylindrical trichogyne, attached spermatium (**arrowhead**) and gonimoblast initial developing from the protuberant side of the carpogonial base (**arrow**); **E**, carposporangia (**arrow**) and erect gonimoblast filaments (**arrowhead**); **F-L**, *Sirodotia delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. (photos taken from sample SJRP 31918 – holotype): **F**, obconical whorls; **G**, primary fascicle; **H**, primary fascicle with spermatangia (**arrow**) and anomalous shape (bent end)




trichogyne (arrowhead); **I**, carpogonium with a mature trichogyne (arrow) showing the basal hemispherical protuberance (double arrow); **J**, carpogonium with anomalous shape (bifurcate) trichogyne (**arrow**); **K**, carpogonium with a sessile cylindrical trichogyne (short arrow), attached spermatium (long arrow) and gonimoblast initial developing from the protuberant side of the carpogonial base (double **arrow**); **L**, carposporangia (**long arrow**) and erect (**short arrow**) gonimoblast filaments. Scale bars: A, F, 200 µm; B, C, D, E, I, 20 µm; D, K, 10 µm G, H, 50 µm; L, 25 µm. D, redrawn from Johnston *et al*. (2014); K, redrawn from Necchi (1991).

*Revised description*
Plants monoecious or dioecious; whorls 162-364 µm in diameter; primary fascicles (5-)6-10 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.

*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, Appendices 4-6). In addition, they are genetically divergent. In the description of this species from Africa (Madagascar and Reunion) by Skuja (1931) and then later by Necchi *et al.* (1993) in specimens from North America (Arizona and Texas), this species was reported to have spermatangia arranged in clusters. However, this type of arrangement, characterized by the terminal and subterminal cells bearing two to four spermatangia, was not confirmed in the type or protologue. That arrangement of spermatangia in clusters was observed only in *S. assamica*, whereas in some populations of *S. huillensis* only abundant and densely arranged spermatangia were found. 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 the specimen referred to as *S.* aff. *huillensis* collected in South Africa was genetically distinct from specimens described as *S. huillensis* from North America. Therefore, the specimen, *S. aff. huillensis,* collected closer to type locality of *S. huillensis*, is interpreted as *S. huillensis* and the North American specimens represent another species (*S. aquiloamericana*).

***Sirodotia kennedyi*** A.L.Szinte, J.C.Taylor & M.L.Vis
(Fig. 6G-I)

*Phycologia* 194 (2020).

*S. masoalensis* E. Fischer, D. Killmann & D. Quandt, *Plant and Fungal Systematics* 65: 164 (Fischer *et al.* 2020).

Type. — Coll. M.P. Kennedy, 07.VII.2011 (holo-, SANDC[SANDC 19-566]; iso-, BHO[BHO A-0946]).

Type locality. — Zambia, Mutinondo River, Mutinondo Wilderness, 12°16.101'S, 31°22.869'E.

Distribution. — Africa: Zambia and Madagascar.

Representative DNA sequences. — COI-5P (MT109276), *rbc*L (MN974518, MT109266).

*Description*
Plants dioecious; whorls 115-315 µm in diameter; primary fascicles, 3-5 cells; proximal cells cylindrical or ellipsoidal; distal cells obovoidal or ellipsoidal; secondary fascicles present, covering half to the entire internode; spermatangia spherical, on primary or secondary fascicles, 6-8 µm in diameter; 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 or ellipsoidal, 9-10 µm in length, 5-7 µm in diameter.

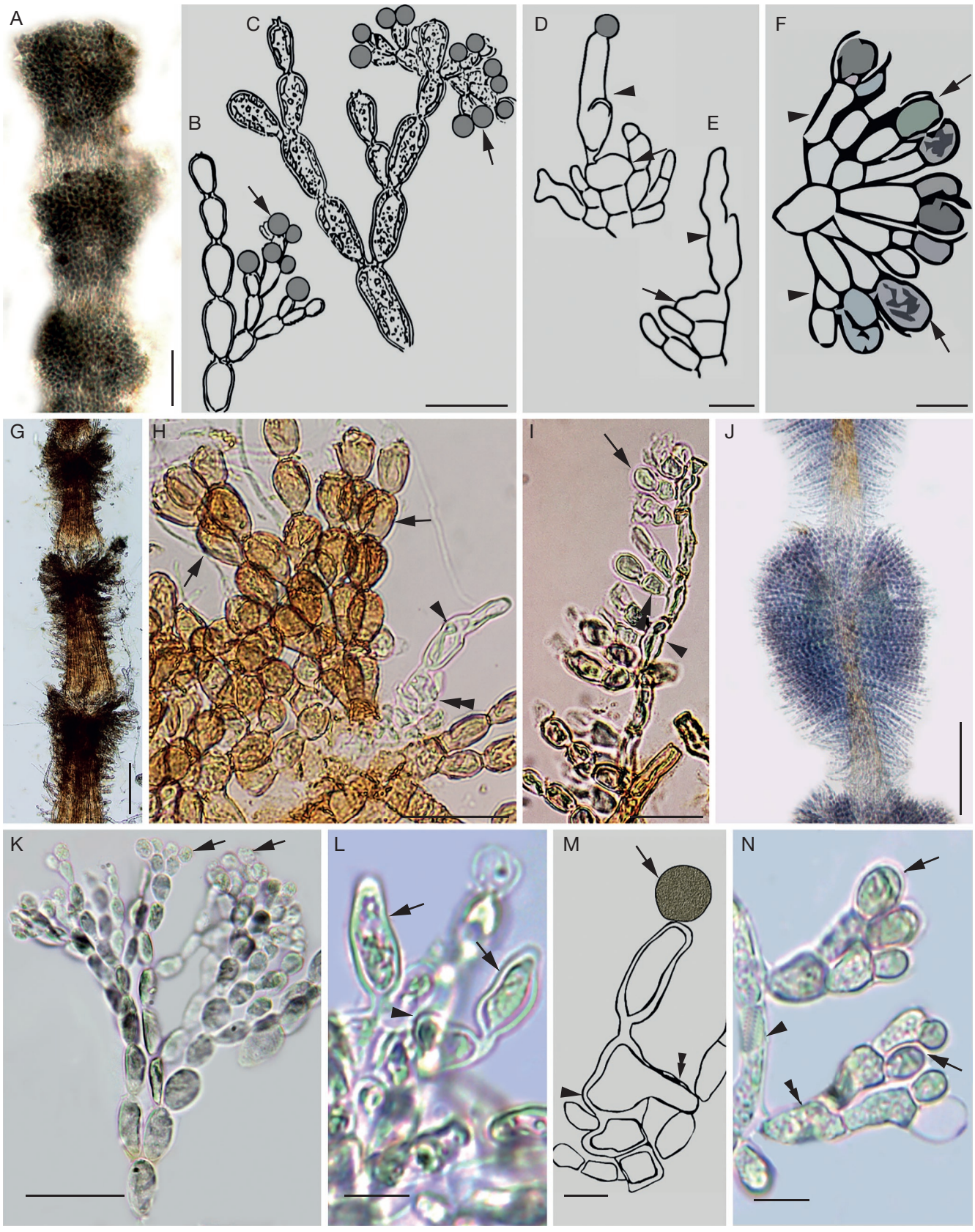
*Remarks*
This species was recently described by Szinte *et al.* (2020) and it is most closely comparable to *S. huillensis* based on the reduced whorls (162-364 µm in diameter), shorter carposporangia (8-10 µm in length, respectively) and the occurrence in Africa. *Sirodotia kennedyi* differs from *S. huillensis* in number of primary fascicle cells, 3-5 versus 5-9(-10), respectively. In addition, they are genetically divergent. The recently described species *S. masoalensis* did not differ from *S. kennedyi* in morphology and DNA sequence and is here proposed as a synonym.

***Sirodotia suecica*** Kylin
(Fig. 6J-N)

*Nova Acta Regiae Societatis Scientiarum Upsaliensis*, ser IV, 3: 38 (Kylin 1912). — *Batrachospermum suecicum* (Kylin) Necchi & Entwisle, *Phycologia* 29: 486 (1990).

*Sirodotia fennica* Skuja, *Archiv für Protistenkunda* 74: 297 (1931).

Fig. 6. — Morphological features of *Sirodotia* species: **A**-**E**. *Sirodotia huillensis* (Welwitsch ex West & G.S.West) Skuja (photos taken from sample BHO A-1447): **A**, obconical whorls; **B**, **C**, primary fascicles with spermatangia (**arrows**); **D**, **E**, carpogonia with irregular trichogyne (**arrowheads**) showing the basal hemispherical protuberance (**double arrows**) and gonimoblast initial developing from the protuberant side of the carpogonium (**arrow**); **F**, carposporangia (**arrows**) and erect gonimoblast filaments (**arrowheads**). **G**-**I**, *Sirodotia kennedyi* (photos taken from sample BHO A-0946): **G**, obconical whorls; **H**, primary fascicles (**arrows**) and carpogonium with irregular trichogyne (**arowhead**) and basal hemispherical protuberance (**double arrowhead**); **I**, carposporangia (**arrow**), prostrate (**arrowhead**) and erect (**double arrowhead**) gonimoblast filaments. **J**-**N**, *Sirodotia suecica* Kylin (photos taken from samples BHO A-0266, SJRP32582, SJRP32583):




**J**, obconical whorls; **K**, primary fascicles with terminal spermatangia (**arrows**); **L**, carpogonia with mature trichogyne (**arrows**) and basal hemispherical protuberance (**arrowhead**); **M**, fertilized carpogonium with attached spermatium (**arrow**) and gonimoblast initial (**double arrowhead**) on the opposite side of the basal protuberance (**arrowhead**); **N**, carposporangia (**arrows**), prostrate (**arrowhead**) and erect (**double arrowhead**) gonimoblast filaments. Scale bars: A, G, J, 100 µm; B-C, H-I; D-F, L-M, 10 µm; K, 50 µm. B-F, redrawn from Skuja (1931); H-I, modified from Szinte *et al*. (2020); M, redrawn from Necchi *et al*. (1993).

*Sirodotia 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. — H. Kylin, 3.VIII.1909 (lecto-, LD; isolecto-, UPS[UPS A-653877]).

Type locality. — Sweden, Skäne, Osby; 56°23'01"N, 13°59'34"E.

Additional specimen examined. — BHO A-0264, SJRP 32581, SJRP 32582, SJRP 32583, NSW799516, MEL2033246A, 2268154A, WELT A027220 (Appendix 1).

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

Representative DNA sequences. — COI-5P (MW053472, MW053473, MW053474), *rbc*L (MW053484, JF344724, JF344725) and LSU (MW053504, MW053505).

*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.

*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* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. , *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. 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 clearly 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 when possible, 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 useful due to the variation observed across this species. 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). 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*). We conclude that *S. segawae*, *S. yutakae* and *S. sinica* should be placed in synonymy with *S. suecica*. Overall, we accept only one taxon including these other names as synonyms, that is characterized by the gonimoblast initial developing from the opposite side of the basal protuberance of carpogonium.

Doubtful species

The status of the species listed below could not be confirmed due to the unavailability of type specimens and/or of descriptions that lack the primary diagnostic characters required for species identification. They all have a very limited geographic distribution, some known only by type localities.

***Sirodotia cirrhosa***
Skuja ex M.S.Balakrishnan & B.B.Chaugule

*Indian Batrachospermaceae*: 242 (1980).

Type specimen not available for analysis.

***Sirodotia gardneri*** Skuja ex L.Flint

*American Journal of Botany* 37: 754 (1951).

Type specimen is not available for analysis. The type specimen analyzed by Necchi *et al.* (1993) was a male plant and therefore key characteristics could not be confirmed.

***Sirodotia huangshanensis*** Z.X.Shi & S.L.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 distinguishing 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.

## 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 valid species names were listed in the genus before this study (Ott 2009; Guiry & Guiry 2020), of which 14 were currently accepted, three of them 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 & Necchi 2013). Three of those accepted species (*S. cirrhosa*, *S. gardneri* and *S. huangshanensis*) could not be confirmed here as separate taxa because the type specimens were not available for analysis or the characters used to describe them were not of taxonomic value. Three species (*S. segawae*, *S. sinica* and *S. yutakae*) are here proposed as synonyms of *S. suecica,* and *S. ateleia* is considered a synonym of *S. delicatula.* Four species (*S. acuminata*, *S. fennica*, *S. goebelii* and *S. tenuissima*) already synonymized with *S. suecica*, are accepted as such here. We therefore recognize three relatively well-known and widely reported species (*S. delicatula*, *S. huillensis*, *S. suecica*) and two recently described species (*S. assamica* and *S. kennedyi*). A third species described recently (*S. masoalensis*) is here proposed as a synonym of *S. kennedyi* based on similar morphology and DNA sequence data.

Four additional *Sirodotia* species were here proposed as new: *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. aquiloamericana*, *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. Some of the species are reported from a single continent or restricted regions and thus biogeography can, and indeed must, be used along with morphology for identification if DNA sequence data are not available. The following species exhibited a well-defined geographical pattern: *S. delicatuliformis* sp. nov. from Brazil and Costa Rica, *S. amazonica* sp. nov. (midwestern and norther region of Brazil) and *S. cryptica* sp. nov. (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 & Necchi 2014; Rossignolo & Necchi 2016; Necchi *et al.* 2018, 2019a, b; Vis *et al.* 2020). The interspecific divergence values for *rbc*L and COI-5P were relatively high (1.6-7% 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 lineages in the phylogenetic tree.

As mentioned earlier in this paper, all collections 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, our study supports the findings of Paiano & Necchi (2013) that there are cryptic species of *Sirodotia* in Brazil based on genetic data. Johnston *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 also identified as *S. delicatula*. The sequence from Malaysia is now considered to be *S. delicatula*. Therefore, while *S. delicatula* is no longer part of the Brazilian algal flora, we recognize three new species from that country: *S. amazonica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov., *S. cryptica* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov. and *S. delicatuliformis* Necchi, N.L.Rossignolo & M.O.Paiano, sp. nov.

The diagnostic morphological characters used to distingish 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 (Appendices 4-6). Another character applied by Kumano (2002) to distinguish species in the genus (carpogonium curved or deflexed to distinguish *S. goebelii*; taking this from the protologue) has 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 order Batrachospermales because polygamy is too 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). However, in some cases, substancial differences in the DNA sequence data and geography alone have been used to distinguish taxa. Overall, the results highlight a pattern of high genetic diversity in *Sirodotia* and a new understanding of species richness and biogeography.

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# APPENDICES

APPENDIX 1. — 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 | rbcL | LSU |
| South America/ Brazil | | | | |
| *S. delicatuliformis* sp. nov. 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 | KC951855A | – |
| *S. delicatuliformis* sp. nov. 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). | MW053460 | KC951856A | MW053485 |
| *S. delicatuliformis* sp. nov. 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 | KC951857A | MW053486 |
| *S. delicatuliformis* sp. nov. 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.VII.2008 (SJRP 31918). | KF010486 | KC951858A | MW053487 |
| *S. delicatuliformis* sp. nov. BR5 | Brazil: São Paulo, Américo de Campos, Ribeirão Águas Paradas, 20°17'41"S, 49°46'35"W; O. Necchi Jr. 1.VI.2009 (SJRP 31919). | KF010483 | KC951859A | MW053488 |
| *S. delicatuliformis* sp. nov. BR6 | Brazil: São Paulo, São José do Rio Preto, stream Lagoa, 49°20'00"W, 20°49'00"S; O. Necchi Jr. & Branco, L.H.Z, 27.VIII.1999 (SJRP 23450). | – | KC951860A | – |
| *S. delicatuliformis* sp. nov. 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). | MW053461 | KC951861A | – |
| *S. delicatuliformis* sp. nov. BR8 | Brazil: Espirito Santo, Domingos Martins, Pedra Azul, Estrada Rota do Lagarto, 20°24'49"S, 41°02'23"W; O. Necchi Jr., 5.VII.2009 (SJRP 31920). | KF010487 | KC951862B | MW053490 |
| *S. delicatuliformis* sp. nov. 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 | KC951863B | – |
| *S. delicatuliformis* sp. nov. 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 | KC951864B | – |
| *S. delicatuliformis* sp. nov. BR11 | Brazil: Espirito Santo, Marechal Floriano, BR-262, km 73-74, entrada para "Parque do China", 20°24'39"S, 40°52'48"W; O. Necchi Jr. 20.VII.06 (SJRP 32156). | MW053462 | MW053478B | – |
| *S. cryptica* sp. nov. BR12 | Brazil: Goias, Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, 14°09'58"S, 47°50'39"W; O. Necchi Jr., 6.IV.2010 (SJRP 31923). | KF010488 | KC951865 | MW053494 |
| *S. cryptica* sp. nov. BR13 | Brazil: Goias, Rodovia GO-070, entre os municípios de Jussara e Itaberaí, 16°00'32,7"S, 48°55'55, 9"W; F.R. Borges, 15.IV.2014 (SJRP 32575). | MW053463 | MW053479 | MW053495 |
| *S. amazonica* sp. nov. BR14 | Brazil: Mato Grosso, Baixada Morena, Rio Roquete, BR-163, sentido Santarém, 11°32'20"S, 55°31'14"W; 19.IX.2010 (SJRP 32576). | KF010490 | KC951867 | MW053496 |
| *S. amazonica* sp. nov. 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 | MW053497 |
| *S. cryptica* sp. nov. 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 | MW053498 |
| *S. amazonica* sp. nov. BR17 | Brazil: Mato Grosso, Sinop, BR-163, 14 km do trevo da cidade, 12°00'37"S, 55°31'01"W; O. Necchi Jr. *et al.*, 03.VI.2013 (SJRP 32577). | MW053464 | MW053480 | MW053499 |
| *S. amazonica* sp. nov. BR18 | Brazil: Mato Grosso, Sinop, BR-163, 34 km do trevo da cidade; 11°36'05"S, 55°25'41"W; O. Necchi Jr., F.R. Borges & A.S. Garcia Fo., 04.VI.2013 (SJRP 32578). | MW053465 | MW053481 | MW053500 |
| *S. delicatuliformis* sp. nov. BR19 | Brazil: Minas Gerais, Parque Nacional da Serra do Cipó, CIPÓ 3 - 25.VII.2003 (SJRP 32579). | MW053466 | MW053482 | MW053501 |
| *S. delicatuliformis* sp. nov. BR20 | Brazil: Minas Gerais, Parque Nacional da Serra do Cipó, CIPÓ 8 - 07.VIII.2003 (SJRP 32580). | MW053467 | MW053483 | MW053502 |
| *S. amazonica* sp. nov. BR21 | Brazil, Roraima, Mucajaí, estrada para Roxinho, próximo ao trevo, 02°25'51"S, 61°02'23"W; O. Necchi Jr. *et al.*, 5.VII.2013 (SJRP 32139, 32140). | – | – | MW053503 |
| North America | | | | |
| *S. aquiloamericana* sp. nov. MEX1 | México: Rt. 120 in Jalpar. 24.IV.1997. No voucher specimen. | MW053468 | AF126414 | – |
| *S. aquiloamericana* sp. nov. US1 | United States: Outlet canal of Montezuma Well, Arizona, 34°38'57"N, 111°45'08"W; T.A. Dempster. 24.IV.2011. (BHO A-0437, MICH672226). | MW053469 | JN408523 | – |
| *S. aquiloamericana* sp. nov. US2 | United States: Barton Spring, Austin, Texas, 30°15'50"N, 97°46'17"W; M.L. Vis & E. Johnston. 16.XII.2010. (BHO A-0410, MICH672225) | MW053470 | JF344716 | – |
| *S. delicatuliformis* sp. nov. CR1 | Costa Rica: Rt. 16 *c.* 8km south of San Vito. 18.II.1998. No voucher specimen | MW053471 | AF126410 | – |
| Africa | | | | |
| *S. huillensis* AS1 | South Africa: X31-5 No. 1, Sabie River Catchment. Gerhard Strydom. IV.2000. (BHO A-1447, MICH672222). | This study | JF344717 | – |

Appendix 1. — Continuation

| Species/Code | Locality, collector, collection date, and herbarium voucher. | GenBank number COI-5P | rbcL | LSU |
|---|---|---|---|---|
| *S. suecica* AS2 | South Africa: Tributary of the Bot River off Van der Stel Pass Road near Cape Town; 34°09'31.5"S, 19°13'33"E; M.L. Vis, W.B. Chiasson & G. Ellis. 22.VIII.2005. (BHO A-0264, MICH672232). | EU636738 | JN408524 | – |
| Europe | | | | |
| *S. suecica* FI1 | Finland: Soutujoki, Housukoski, (FI-8), 62°29'20"N, 24°41'32"E; P. Eloranta. 12.III.2007. | MW053472 | – | MW053504 |
| *S. suecica* FI2 | Finland: Meskusjoki, Kuusamo, (FI-15), 65°58'22"N, 29°08'11"E; P. Eloranta. 15.VII.2012. | – | MW053484 | MW053505 |
| *S. suecica* FI3 | Finland: Kärkelänjoki River, Karjulankoski Rapid, (isolate 3) FI-18, 60°16'18"N, 23°35'49"E; P. Eloranta. 23.VI.2009. (MICH672231). | – | JF344722 | – |
| *S. suecica* FI4 | Finland: Tarhapäänkoski, Horhanvirta River, (isolate 5) FI-22, 62°20'29"N, 24°44'05"E; P. Eloranta. 16.VII.2010. (MICH672235). | MW053473 | JF344724C | – |
| *S. suecica* FI5 | Finland: Uittamonkoski Rapid, outlet of Lake Uittamonjärvi, (isolate 6) FI-23. P. Eloranta. 16.VII.2010. (MICH672234). | MW053474 | JF344725D | – |
| *S. suecica* FI6 | Finland: Likojoki, Kuusamo, (FI-31), 65°47'12"N, 30°02'52"E; P. Eloranta. 17.VII.2012. | MW053475 | – | – |
| Asia | | | | |
| *S. assamica* IN1a | India: Assam, Nagaon District, Chapanala; 26°19'13.7"N, 92°10'16.5"E; O. Necchi Jr., J.A. West, E.K. Ganesan & F. Yasmin, 23.II.2018. (SJRP 32583). | MN508239 | MN496129 | MW053506 |
| *S. assamica* IN1b | India: Assam, Nagaon District, Chapanala; 26°19'13.7"N, 92°10'16.5"E; F. Yasmin, 25.II.2019. (SJRP 32584). | – | – | – |
| *S. assamica* IN2 | India: Assam, Sonapur, Tegheria; 26°05'52"N, 92°02'02"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 Amour, 27. 2011. (BHO A-0984, KEP 216669, KLU PSM12138). | This study? | KF557560 | MW053507 |
| Australasia | | | | |
| *S. suecica* AU1 | Australia: Dom Dom Creek, Anderson Road crossing, *c.* 1 km west of Naberthong, Victoria, 37°33'54"S, 145°39'08"E; T.J. Entwisle (3394), 05.I.2010. (NSW799516). | MW053476 | JF344715 | MW053508 |
| *S. suecica* AU2 | Australia: Nigger Creek, Herberton, Queensland; 17°23'00"S, 145°23'00"E; T. J. Entwisle (2430), 03.VII.1994 (MEL2033246A, 2268154A). | MW053477 | AF209977 | This study |
| *S. suecica* NZ1 | New Zeland: Wanganui River, Beach track at the end of La Fontaine Road, 43°07'48"S, 170°36'37"E; M. L. Vis. 04.XII.2005 (BHO A-0265, MICH672229, WELT A027220, A027221). | – | JF344719 | MW053509 |

APPENDIX 2. — Sequences of *rbc*L, COI-5P and LSU *Sirodotia* species obtained from Genbank used in the analyzes.

| Species | Locality, collector, collection date, and herbarium voucher. | GenBank Number *rbc*L | COI-5P | LSU | Reference |
|---|---|---|---|---|---|
| *S. aquiloamericana* sp. nov. | United States: San Marcos, San Marcos river, Texas. 1.XII.1993. | AF029157 | | EU636739 | Vis *et al.* (1998) Sherwood *et al.* (2010) |
| *S. kennedyi* | Africa: Zambia, Mutinondo River, 12.3911'S, 31.3257'E, M. P. Kennedy. 7.VII.2011 (SANDC 19-566, BH0 A-0946 | MN974518 | | | Szinte *et al.*(2020) |
| *S. suecica* | United States: Rhode Island, Chipuxet River at Rt. 138, West Kingston. 27.VI.1992 | AF029158 | | | Vis *et al.* (1998) |
| *S. suecica* | United States: Town Road. in Coventry, RI. 18.IV.1992. | AF126420 | | | Vis & Sheath. (1999) |
| *S. aquiloamericana* sp. nov. | United States: New Hampshire, near Enfield, 43°38′N, 72°09′W. | JF701687 | | | Necchi & Oliveira (2011) |
| *S. delicatuliformis* sp. nov. | Brazil: Minas Gerais, Parque Nacional da Serra do Cipó, Rio Alto do Palácio, 100m da rodovia MG-010. 19°17'00"S, 43°33'00"W, 25.VII.2003. | DQ646474 | | | Necchi *et al.* (2007) |
| *S. delicatuliformis* sp. nov. | Brazil: São Paulo, São José do Rio Preto, Rio Lagoa, 20°49'35"S, 49°21'37"W, isolado de cultura. | DQ646475 | | | Necchi *et al.* (2007) |
| *S. delicatuliformis* sp. nov. | Brazil: São Paulo, São José do Rio Preto, Rio Macaco, 20°50'31"S, 49°21'07"W, isolado de cultura. | DQ646476 | | | Necchi *et al.* (2007) |
| *S. delicatuliformis* sp. nov. | Brazil: São Paulo, São José do Rio Preto, Rio Talhadinho, 20°43'24"S, 49°18'21"W, 07.x.2004. | DQ646477 | | | Necchi *et al.* (2007) |
| *S. delicatuliformis* sp. nov. | Brazil: São Paulo, Américo de Campos, Rio Aguas Paradas, 20°18'00"S, 49°46'02"W, 06.X.2004. | DQ646478 | | | Necchi *et al.* (2007) |
| *S. suecica* | United States: Canada, Labrador, 33 Km west of Goose Bay on road Churchill Falls. 30.VII.1993. | AF126407 | | | Vis & Sheath. (1999) |
| *S. suecica* | United States: Big Beaver Creek, at US 176 bridge crossing, Calhoun Co., South Carolina. E.K. Hollingsworth. 16.V.2008 (BHO A-0268, MICH). | JF344718 | KM055337 | KM055260 | Lam *et al.* (2012) |
| *S. suecica* | United States: 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 (MICH). | JF344720 | | - | Lam *et al.* (2012) |
| *S. suecica* | Finland: Nytkymenjoki River, Hasakoski Rapid, (isolate 2) FI-10. P. Eloranta. 07.VIII.2008 (MICH). | JF344721 | | | Lam *et al.* (2012) |
| *S. suecica* | Finland: Kontysjoki Upper River, (isolate 4) FI-4. P. Eloranta. 16.VI.2010 (MICH) | JF344723 | | | Lam *et al.* (2012) |

APPENDIX 3. — Outgroup sequences used in *rbc*L, COI and LSU concatenated alignment for phylogenetic analyses.

| | GenBank Number | | |
|---|---|---|---|
| **Taxon** | **rbcL** | **COI-5P** | **LSU** |
| Batrachospermales | | | |
| *Acarposporophycos brasiliensis* | FJ386458 | KU672388 | – |
| *Batrachospermum dapsile* | KM593855 | – | – |
| *Batrachospermum gelatinosum* | GU810833 | KM055327 | KM055251 |
| *Batrachospermum gelatinosum* | KJ825965 | – | – |
| *Batrachospermum helminthosum* | AB114642 | – | – |
| *Batrachospermum helminthosum* | AY198419 | – | – |
| *Batrachospermum naiadis* | KM593857 | – | – |
| *Batrachospermum pozoazulense* | KM593858 | – | – |
| *Batrachospermum shanxiense* | KM593851 | – | – |
| *Batrachospermum turfosum* | AY423408 | – | – |
| *Batrachospermum turfosum* | AF029147 | – | – |
| *Batrachospermum viride-brasiliense* | KM078048 | – | – |
| *Kumanoa ambígua* | GQ368885 | – | – |
| *Kumanoa cipoensis* | GQ368887 | – | – |
| *Kumanoa gracílima* | JN590013 | – | – |
| *Kumanoa holtonii* | JN590004 | JN604921 | |
| *Kumanoa louisianae* | JN590005 | JN604924 | |
| *Kumanoa procarpa* | FJ386464 | KM055332 | KM055256 |
| *Lemanea bourelis* | 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 | – | – |
| *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 | – |

APPENDIX 4. — Morphological characters of *Sirodotia* specimens examined in this study or reported in relevant works. Sample code according to Table S1. For numerical data, the values (µm) represent the amplitude of variation (upper line) and mean ± standard deviation (lower line).

| | | | | | | Primary fascicles | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Whorl | | | number | Proximal | | Distal | | | |
| Species | Separation of sex | Diameter | Arrange | Internode length | of cells | 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. 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 |
| *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 (1938b) | Dioecious | 360 | – | – | 5-8 | Cylindrical, 23 x 11 | Ellipsoidal, | 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* sp. nov. 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 (1938b) | 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) | – | – | – | – | – | – |
| *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 | 217-230a (224 ± 5) | Open | 209-360a (275 ± 67) | 7-9a (8 ± 0.7) | Cylindrical, Obovoidal 16-22a (18 ± 2,1) | Cylindrical, Obovoidal 7-8a (7 ± 0,4) | 8-11a (9 ± 1.5) | 5-7a (6 ± 0.9) | Ellipsoidal, Obovoidal | 1.5-1.7 (1.6 ± 0.1) |
| Skuja (1931) | Monoecious | 400 | – | – | – | Cylindrical, Obovoidal 12-32 | Cylindrical, Obovoidal 3-8 | – | – | 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* Xie & Shi (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 | – |

Appendix 4. — Continuation

| Species | Separation of sex | Whorl | | Internode length | Primary fascicles | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | number of cells | Proximal | | Distal | | | |
| | | Diameter | Arrange | | | Length | Diameter | Length | Diameter | Shape | L/D |
| Necchi *et al.* (1993) | Monoecious | 162-278 (214) | – | – | 6-10 (7,4) | – | – | – | – | – | – |
| S. kennedy | 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, Obovoid 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 (1982) | 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) |

Appendix 5. — Reproductive characters of *Sirodotia* specimens examined in this study or reported in relevant works. Sample code as Table S1. For numerical data the values (µm) represent amplitude of variation (upper line) and mean ± standard deviation (lower line).a. N= 2-5; 1. According Flint (1950); 2. According Lam *et al.* (2012); 3. According Kumano (1982).

| | Carpogonium | | | Number of cells in the carpogonial branch | Carposporangia | | | Number of erect gonimoblastic filament cells | Spermatangia | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Length** | **Diameter** | **Shape** | | **Length** | **Diameter** | **Shape** | | **Diameter** | **Shape** |
| *S. acuminata* Necchi *et al.* (1993) | 19-37 (28.1) | 5-9 (6.1) | Cylindrical-elongated, 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) | Cylindrical-elongated, Fusiform, wavy margins | 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 |
| *S. assamica* Rossignolo *et. al*. (2020) | 37-64 (51.7 ± 7.3) | 10-16 (11.6 ± 1.4) | Cylindrical-elongated, 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) | Cylindrical-elongated | – | – | – | – | – | – | – |
| Skuja (1938b) | 45-70 | – | Cylindrical-elongated, Conical-elongated | 2-4 | – | – | – | – | – | – |
| *S. cirrhosa* Balakrishnan & Chaugule (1980) | 29-41 | 8-10 | Cylindrical-elongated | 3-5 | 8-11 | 5.5-8 | Ellipsoidal, Obovoidal | – | 6,5-7,5 | Subspherical |
| *S. cryptica* sp. nov. This study (31923, 31925, 32575) | 24-58 (42.7 ± 8.3) | 9-15 (10.5 ± 1.4) | Cylindrical-elongated, Fusiform, wavy margins | 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 |
| *S. delicatula* This study (MR-08) | 30-57 (38.5 ± 8.7) | 8-13 (10.4 ± 1.2) | Cylindrical-elongated, 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 (1938b) | 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) | Cylindrical-elongated, 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) | Cylindrical-elongated, 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 | Cylindrical-elongated | 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) | 201 | – | Cylindrical-elongated | – | – | – | – | 2-31 | 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 |

Appendix 5. — Continuation

| Species | Carpogonium | | | Number of cells in the carpogonial branch | Carposporangia | | | Number of erect gonimoblastic filament cells | Spermatangia | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Length | Diameter | Shape | | Length | Diameter | Shape | | Diameter | Shape |
| *S. huangshanensis* Xie & Shi (2004) | 48-55 | 10 | Cylindrical-elongated, Ellipsoidal | 2-6 | 9-12 | 7-10 | Ellipsoidal | 3-4 | 6-8 | Spherical |
| *S. aff. huillensis* This study (AS1) | 34-422 | – | Cylindrical-elongated, wavy margins | 1-32 | 8a (8.3 ± 0.1) | 5-6a (5.9 ± 0.4) | Obovoidal | 2-4a (3.0 ± 0.8) | 5-7a (5.9 ± 0.7) | Spherical |
| *S. huillensis* Isótipo | – | – | – | – | – | – | – | – | 5-6.5a (5.5 ± 0.6) | Spherical |
| Skuja (1931) | 32-48 | 10-12 | Cylindrical-elongated, 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 | Cylindrical-elongated | – | – | – | – | – | 6-7 | Spherical |
| *S. kennedy* | 25-40 | – | Cylindrical-elongated, club-shaped | 3-4 | 9-10 | 6-7 | Obovoidal | 1-2 | – | – |
| *S. segawae* Isótipo | 30-463 | – | Cylindrical-elongated | 3-93 | 10-20a (14.3 ± 3.8) | 8-11a (9.8 ± 1.1) | Ovoidal, Obovoidal | 3-4 (3.3 ± 0.4) | 6-83 | Spherical3 |
| *S. sinica* Jao (1941) | 36 - 41 | 5.4-6.3 | Cylindrical-elongated, club-shaped | 4-8 | 12-18 | 9-10 | Subspherica | – | 7-9 | Spherical |
| *S. suecica* (FI1, FI2, FI6, AS2, NZ1) | 24-39 (28.5 ± 3.7) | 8-15 (10.7 ± 2.1) | Cylindrical-elongated, 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 |
| Isótipo | 24-25a (24.5 ± 0.5) | 10-11a (10.5 ± 0.5) | Cylindrical-elongated | 2-3a (2.5 ± 0.5) | 11-12a (11.7 ± 0.4) | 7-9a (7.8 ± 0.5) | Obovoidal | 2-3a (2.8 ± 0.4) | – | – |
| Israelson (1942) | 20-26 | – | Cylindrical | 2-5 | 8-12 | 6-8 | Obovoidal | – | 6-7.5 | Spherical, Ellipsoidal |
| Entwisle & Kraft (1982) | 19-42 | 4-8 | Cylindrical, Ellipsoidal, Piriform | 3-5 | 8-10 | 6-8 | Ellipsoidal, Piriform | 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 | – | Ellipsoidal Cylindrical, 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* Isótipo | 20-48a (33 ± 10.9) | 9-11a (9.9 ± 0.6) | Cylindrical, Pear-shaped | 2-4a (3.3 ± 0.8) | 10-14a (11.4 ± 1.6) | 8-10a (8.9 ± 0.6) | Obovoidal | 2-3a (2.5 ± 0.5) | 5-8a (6.3 ± 1.1) | Spherical |

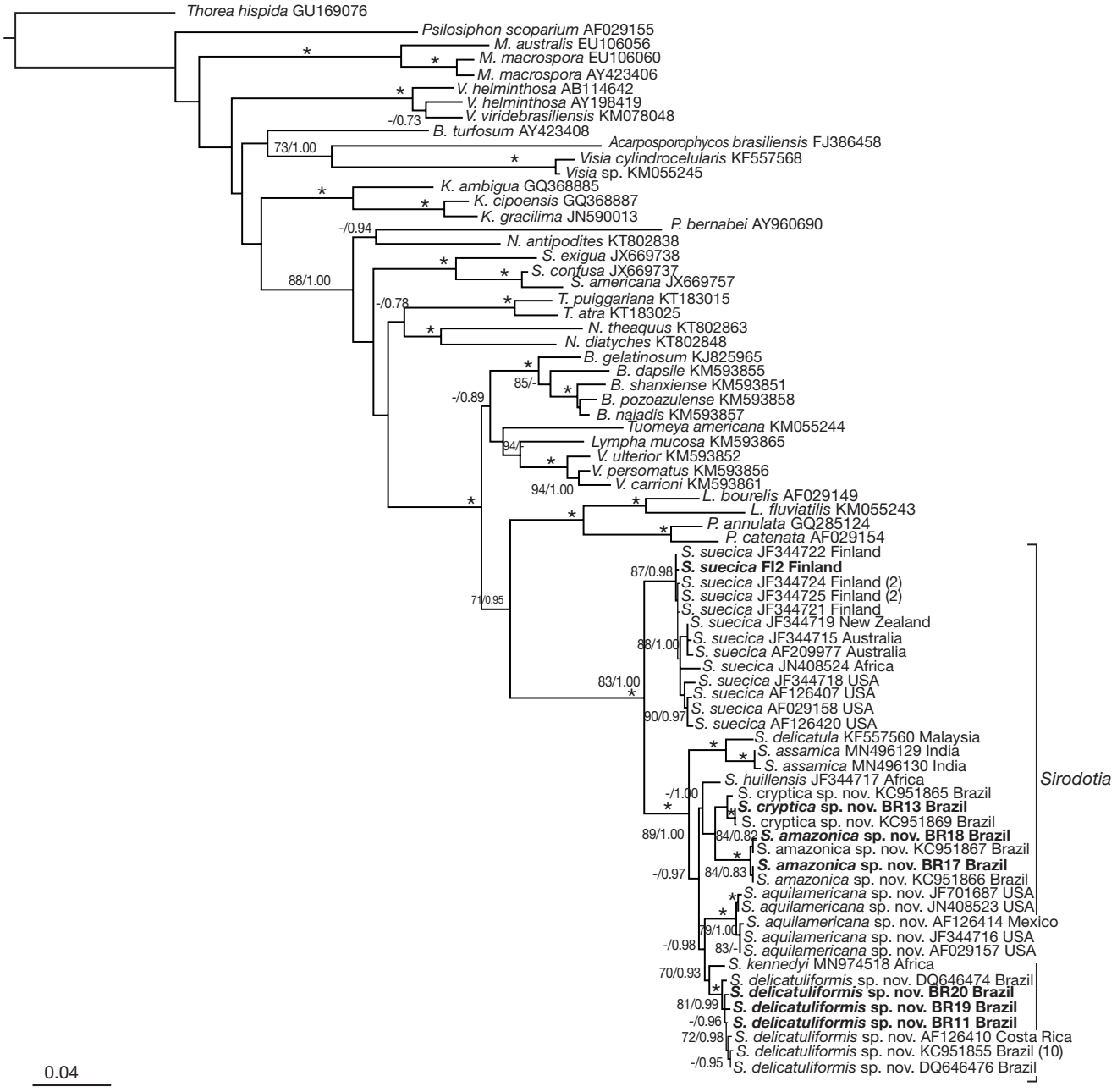
APPENDIX 6. — Comparative morphological characters and geographic distribution of some species of *Sirodotia*. The dimension data are presented in µm.

| 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: United States | 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, United States | 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 | – | 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 | 20-301 (24.3 ± 4.1) | 29-41 | basal protuberance | Ellipsoidal, Obovoidal 8-11 | 5.5-8 | Asia: India | Balakrishnan & Chaugule (1980) |
| *S. cryptica* sp. nov. | 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) | (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 |
| *S. delicatuiformis* | 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-) 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 |

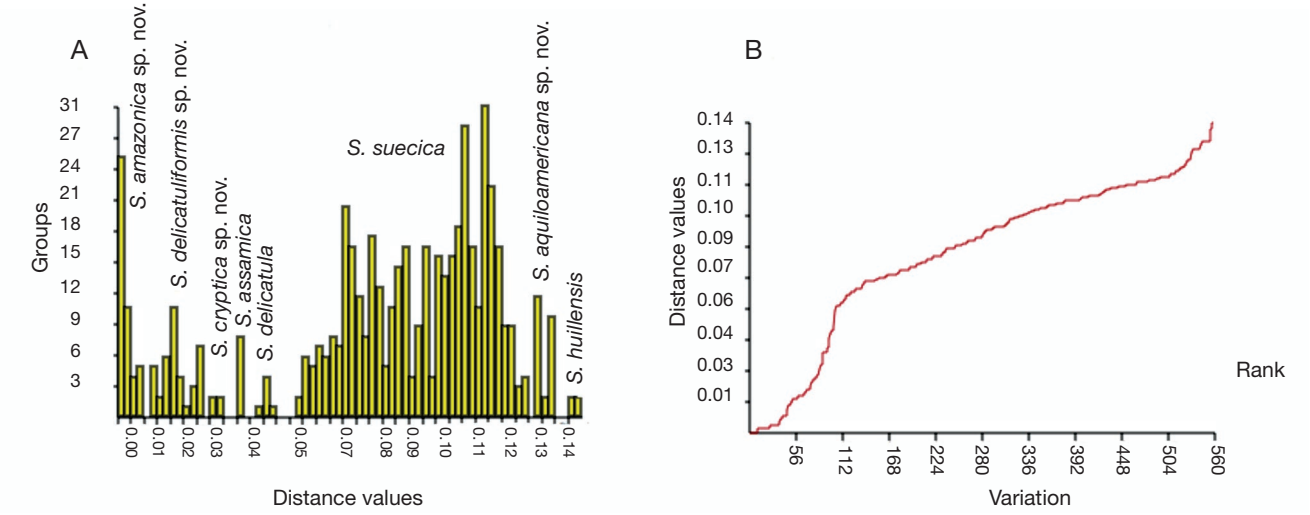
APPENDIX 6. — Continuation

| 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. gardneri* | 288-463 | 6-10 | – | Terminal, single or in pairs, 5-8 | – | 20 | – | – | – | North America: United States | 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 | 10-451 (21.3 ± 12.6) | 48-55 | basal protuberance | Ellipsoidal, 9-12 | 7-10 | Asia: China | Xie & Shi (2004), Hu, H. & Wei, Y. (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) | 5-141 (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 | – | 16-221 (19 ± 2.8) | 25-40 | – | Obovoidal, 9-10 | 6-7 | Africa: South Africa | Szinte *et al.* (2020) |
| *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 | 20-401 (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 em ramos laterais curtos dos fascículos, 5-8 (6.3 ± 1.1) | 10-201 (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 |

Appendix 7. — Maximum likelihood phylogenetic tree based on rbcL. 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 Appendix 1.



Appendix 8. — 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.



Appendix 9. — 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 Appendices 1, 2. Scale represents substitutions per site.