Research Article

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Occurrence of *Dictyosphaeria* (Cladophorales, Chlorophyta) species on the Brazilian coast and oceanic islands, based on morphological and molecular data

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Abstract: Molecular data related to morphology from the multicellular vesiculated genus *Dictyosphaeria* are scarce. We provide the first molecular data on *Dictyosphaeria* species from the southwestern Atlantic Ocean, sampled from the Brazilian northeastern coast, and two oceanic islands (Trindade, southeastern Brazil, and Fernando de Noronha, northeastern Brazil). The samples from Brazil were morphologically identified as *Dictyosphaeria versluysii* and *D. ocellata*. Our newly generated sequences of *D. versluysii* formed a monophyletic clade, while their relationship with *D. versluysii* from the Indo-Pacific Ocean, where the type locality of the species is located, was unresolved. Additionally, the newly generated sequences of *D. ocellata* confirm its status as a distinct species but reinforce the difficulties in morphologically separating this species. These results might be indicative of cryptic diversity in *D. versluysii* and highlight the need for molecular data from other geographic locations to understand the diversity and distribution of *Dictyosphaeria* species.

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1 Introduction

The Cladophorales is one of the most morphologically diverse orders of Ulvophyceae, which includes uniseriate filamentous, laminar, vesiculose (or 'balloon') and reticulate thalli. Currently, the order has 522 described species distributed from polar to tropical waters (Guiry and Guiry 2024), and its taxonomic circumscription is a challenge due to its simple morphology, phenotypic plasticity, lack of diagnostic characters, and cryptic diversity (Boedeker et al. 2016; Gestinari et al. 2009; Leliaert et al. 2009; Sherwood et al. 2019).

The genus *Dictyosphaeria* Decaisne is easily distinguished amongst the marine green algae by its multicellular vesiculated honeycomb-like thallus. Its structure is generated by segregative cell divisions with protoplasm dividing simultaneously into several multinucleated cytoplasmic aggregations, followed by the formation of walled spheres that expand and break the parental wall (Enomoto 1981; Mine et al. 2008; Okuda et al. 1997).

Currently, 11 species of *Dictyosphaeria* are flagged as accepted in AlgaeBase (Guiry and Guiry 2024). However, for the Western Atlantic, only three species are reported: *Dictyosphaeria cavernosa* (Fossrkål) Børgesen, *D. ocellata* (M. Howe) Olsen-Stojkovich and *D. versluysii* Weber Bosse (Wynne 2022). *Dictyosphaeria cavernosa* and *D. versluysii* are often identified on the Brazilian coast (Almeida et al. 2012; Alves et al. 2012; Muniz 1993; Pereira 1974; Pinheiro-Vieira and Ferreira 1968; Széchy et al. 1989), occurring from the northeastern to southeastern coast. *Dictyosphaeria ocellata* is restricted to the oceanic islands Rocas Atoll (northeastern Brazil) (Villaça et al. 2010) and Trindade Island (southeastern Brazil) (Pellizzari et al. 2020). These taxa are distinguished

mainly by thallus shape (sac-like, hemispherical, or crustose), solid or hollow, monostromatic or polystromatic, shape of cells, and presence or absence of spiny-like projections on the inner cell walls (Table 1).

Despite its wide distribution and characteristic morphology, molecular data available for *Dictyosphaeria* species is scarce, and currently, only sequences of *D. cavernosa* and *D. versluysii* are available. Olsen-Stojkovich et al. (1986) used immunological data to suggest that *D. cavernosa* is not monophyletic. Twenty years later, Leliaert et al. (2007), based on DNA sequences of *D. cavernosa* and *D. versluysii*, found large genetic distances among the Indo-Pacific coast specimens of *D. cavernosa* and the ones from the Atlantic and Pacific Oceans analyzed by Olsen-Stojkovich et al. (1986), suggesting cryptic diversity within this taxon. These authors indicated the monophyly of the genus and suggested the need for a broader taxon sampling to better understand phylogenetic relationships within *Dictyosphaeria*, plus the sequencing of material from the type locality of *D. cavernosa* (Saudi Arabia), and of *D. versluysii* (Malaysian archipelago). Herein we provide the first molecular data on *Dictyosphaeria* species from the southwestern Atlantic Ocean, allied to a detailed morphological analysis.

2 Materials and methods

The samples were collected in Alagoas State on the Brazilian northeastern coast (n = 7) and from two oceanic islands, Trindade Island, southeastern Brazil (n = 11), and Fernando de Noronha Island, northeastern Brazil (n = 4) (Table 2). Fragments from specimens were rinsed in water, and cleaned under a stereomicroscope to remove epiphytic algae, with part of this material dried in silica gel for DNA extraction. The remaining material from the same specimens was stored in 4 % formalin or absolute ethanol for morphological analyses. The individuals were morphologically identified using specialized references (Almeida et al. 2012; Alves et al. 2012; Littler and Littler 2000; Tseng and Chang 1962; Weber-van Bosse 1905). Morphological observation and transverse hand sections were made with a razor blade under a stereoscopic microscope (Zeiss, Axioplan, Göttingen, Germany), and anatomical observations were made under an optical microscope (Zeiss, Axioplan, Göttingen, Germany). Micrographs were taken with a digital camera (Canon, SD950IS, Tokyo, Japan) coupled to a microscope. Herbarium vouchers (Table 1) were deposited in Professor Vasconcelos Sobrinho Herbarium (PEUFR) and the University of São Paulo Herbarium (SPF).

DNA extractions were performed according to Doyle and Doyle (1987), excluding RNAse. For phylogenetic analyses, the regions SSU (18S) and LSU (28S) rDNA were amplified by PCR using two overlapping pairs of primers SR1-SS11H and SSU897–18SC2 for SSU, and the primer pair C1FL-D2FL for LSU (Supplementary Table S1), following the cycles

Table 1: Morphological traits of *Dictyosphaeria* species.

Taxon	Thallus	Thallus shape	Cell shape	Spines
Dictyosphaeria australis Setchell	Solid[a]	Slightly flattened[a]	Polygonal[a]	Present, sparse to rare, short[a,b]
Dictyosphaeria cavernosa (Fossrkål) Børgesen	Hollow, monostromatic[c,d,f,m]	Subspherical to irregular, breaking at some points, forming a cavity[c,d]	Polygonal[d]	Absent[c,d]
Dictyosphaeria enteromorpha Montagne *et* Millardet	Hollow[e]	Cylindrical, claviform with constrictions[e]	Polygonal to irregular[e]	Absent[e]
Dictyosphaeria intermedia Weber Bosse	Solid (young), hollow, monostromatic (mature)[f,g]	Spherical to subspherical, never breaking out[f,g]	–	Absent[f]
Dictyosphaeria mutica Yamada	Solid[k]	Spherical to irregular[k]	Polygonal to rounded[k]	Absent[k]
Dictyosphaeria ocellata (M. Howe) Olsen-Stojkovich	Solid[j,l]	Crustose to hemispherical[j,l]	Angular to polyhedral[j,l]	Absent[j,l]
Dictyosphaeria sericea Harvey	Solid[h]	Flattened[h]	Globose-polyhedral[h]	–
Dictyosphaeria spinifera C.K. Tseng *et* Chang	Hollow[j]	Pyriform to irregular[i]	Polygonal[i]	Present, straight to curved[i]
Dictyosphaeria ulvacea Kützing	–	–	–	–
Dictyosphaeria valonioides Zanardini	Hollow, monostromatic	Spherical	Angular to spherical	
Dictyosphaeria versluysii Weber Bosse	Solid, polystromatic[c,d,f,j,m]	Subspherical to slightly flattened[c,d,f,j]	Polygonal[c,d,f,j]	Present, simple, straight or curved[b,c,d,f,j]
Dictyosphaeria ocellata (this study)	Solid	Crustose	Angular to polyhedral	Absent
Dictyosphaeria versluysii (this study)	Solid, polystromatic	Subspherical to slightly flattened	Polygonal	Present, simple, straight

[a]Børgesen (1930), [b]Valet (1966), [c]Leliaert et al. (1998), [d]Alves et al. (2012), [e]Montagne and Millardet (1862), [f]Weber-van Bosse (1905), [g]Egerod (1975), [h]Harvey (1849), [i]Tseng and Chang (1962), [j]Littler and Littler (2000), [k]Yamada (1944), [l]Taylor (1960), [m]Coppejans et al. (2004). (–) data not accessed.

Table 2: Samples from Brazil included in morphological and molecular analysis.

Voucher	Taxon	Locality	Collection date	Collector	Latitude	Longitude
SPF58693	*Dictyosphaeria ocellata*	Trindade Island	23.12.2019	Silva, M.C.S.	20°30′514″S	29°20′220″W
ITLI06	*Dictyosphaeria ocellata*	Trindade Island	05.12.2018	Osaki, V.S.	20°31′249″S	29°19′314″W
SPF58696	*Dictyosphaeria ocellata*	Trindade Island	14.10.2019	Silva, M.C.S.	20°31′249″S	29°19′314″W
SPF58698	*Dictyosphaeria ocellata*	Trindade Island	23.12.2019	Silva, M.C.S.	20°31′552″S	29°18′106″W
SPF58699	*Dictyosphaeria ocellata*	Trindade Island	24.12.2019	Silva, M.C.S.	20°30′492″S	29°18′589″W
SPF58700	*Dictyosphaeria ocellata*	Trindade Island	11.12.2019	Silva, M.C.S.	20°30′217″S	29°18′106″W
SPF58701	*Dictyosphaeria ocellata*	Trindade Island	08.12.2018	Osaki, V.S.	20°31′552″S	29°18′106″W
SPF58702	*Dictyosphaeria ocellata*	Trindade Island	08.12.2018	Osaki, V.S.	20°30′514″S	29°220′220″W
ITCA11	*Dictyosphaeria ocellata*	Trindade Island	08.12.2018	Osaki, V.S.	20°29′491″S	29°19′29″W
SPF58694	*Dictyosphaeria versluysii*	Trindade Island	26.12.2019	Silva, M.C.S.	20°31′249″S	29°19′314″W
ITTA04	*Dictyosphaeria versluysii*	Trindade Island	21.06.2019	Silva, M.C.S.	20°31′552″S	29°18′106″W
PEUFR55634	*Dictyosphaeria versluysii*	Mirante da Sereia, Maceió, Alagoas	18.04.2019	Brito, J.O.F.	9°34′02.3″S	35°38′50.5″W
PEUFR55717	*Dictyosphaeria versluysii*	Riacho Doce, Maceió, Alagoas	19.04.2019	Brito, J.O.F.	9°34′51.9″S	35°39′25.8″W
PEUFR55609	*Dictyosphaeria versluysii*	Pontal do Coruripe, Coruripe, Alagoas	17.05.2019	Brito, J.O.F.	10°9′03.739″S	36°8′05.308″W
PEUFR55596	*Dictyosphaeria versluysii*	Sonho Verde, Paripueira, Alagoas	02.08.2019	Brito, J.O.F.	9°27′7.25″S	35°31′48.023″W
PEUFR55619	*Dictyosphaeria versluysii*	São Miguel dos Milagres, Alagoas	04.08.2019	Brito, J.O.F.	9°16′7.901″S	35°21′59.882″W
PEUFR55687	*Dictyosphaeria versluysii*	São Miguel dos Milagres, Alagoas	04.08.2020	Brito, J.O.F	9°16′7.901″S	35°21′59.882″W
PEUFR55618	*Dictyosphaeria versluysii*	São Miguel dos Milagres, Alagoas	04.08.2021	Brito, J.O.F	9°16′7.901″S	35°21′59.882″W
PEUFR55549	*Dictyosphaeria versluysii*	Barreiras do Boqueirão, Japaratinga, Alagoas	30.08.2019	Brito, J.O.F	9°7′29.809″S	35°16′37.142″W
PEUFR55557	*Dictyosphaeria versluysii*	Barreiras do Boqueirão, Japaratinga, Alagoas	30.08.2020	Brito, J.O.F	9°7′29.809″S	35°16′37.142″W
SPF58695	*Dictyosphaeria versluysii*	Praia de Atalaia, Fernando de Noronha Island, Pernambuco	02.06.2022	Silva, M.C.S.	3°51′264″S	32°24′325″W
SPF58692	*Dictyosphaeria versluysii*	Sueste, Fernando de Noronha Island, Pernambuco	02.06.2022	Silva, M.C.S.	3°52′624″S	32°25′184″W
SPF58703	*Dictyosphaeria versluysii*	Praia do Cachorro, Fernando de Noronha Island, Pernambuco	02.08.2019	Pellizzari, F.	3°50′352″S	32°24′393″W

described by Leliaert et al. (2007). PCRs were performed in 25 µl final volume: 1× PCR *buffer*, 3.0 mM of $MgCl_2$, 1.2 mM of betaine, 1.6 mM of dNTP, 0.4 mM of each primer, 0.31 U of *Taq* DNA polymerase (Promega Corp., Madison, Wisconsin, USA) and 1 µl of extracted DNA. All PCR products were checked by electrophoresis in 1 % agarose to verify product size and were purified using an Illustra GFX PCR DNA and Gel Purification Kit (GE Healthcare, Buckinghamshire, UK), following the manufacturer's instructions. Amplicons for both markers were sequenced using the same primers from PCR and Big Dye Terminator Cycle Sequencing Ready to Reaction Kit (Applied Biosystems, Foster City, USA) on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

Forward and reverse sequences were assembled using Geneious® (2022.1) and compared to sequences in GenBank using the BLAST tool. The generated sequences and sequences acquired in GenBank were analyzed in two datasets, the LSU and SSU rDNA datasets. The LSU data included 48 sequences of 34 taxa, while SSU included 33 sequences of 25 taxa (Supplementary Table S2). The datasets were aligned using ClustalW (Larkin et al. 2007) implemented in MegaX (Kumar et al. 2018). The best evolution model (GTR + I + G) was calculated and selected using jModeltest v2.1.10 under the AIC criterion (Darriba et al. 2012). Maximum Likelihood (ML) analyses were performed in IQ-Tree (Nguyen et al. 2015), with 1000× bootstrap replicates. Bayesian Inference was performed with MrBayes v.3.2.2 (Ronquist et al. 2012), in two runs and four MCMC chains, and 5,000,000 generations sampled every 1000 generations, starting with a random tree, with *burn-in* verified in the software Tracer v1.7 (Rambaut et al. 2018). Percent distances within and between species from both gene matrices were calculated in MEGA X (Supplementary Tables S3 and S4).

3 Results

3.1 Morphological data

All samples from Alagoas State and Fernando de Noronha Island were morphologically identified as *Dictyosphaeria versluysii*, which is in accordance with the descriptions provided by Weber-van Bosse (1905), Leliaert et al. (1998), Littler and Littler (2000) and Alves et al. (2012). All individuals presented a solid polystromatic thallus, with polyhedral cells attached to each other by hapteroidal cells in alternate or opposite rows and presenting inner cell walls with spiny-like projections (Figure 1). The sample SPF58694 (PP455255) from Trindade Island was composed of a few reduced individuals up to 5.38 mm in diameter, lacking inner cell wall spines, and it was initially identified as *D. ocellata*.

Eight samples from Trindade Island were identified as *D. ocellata*, and presented a solid crustose polystromatic thallus, with polyhedral to angular cells attached to each other by simple, bi-trifurcated hapteroidal cells in alternate or opposite rows lacking spiny-like projections in the inner cell wall, attached to substrata by cylindrical cells (Figure 2). In general, these morphological features are in agreement with those described by Howe (1920), Taylor (1960), and Littler and Littler (2000), however, our samples were composed of smaller agglomerates, 3.3–20 mm in diameter versus 10–20 cm in diameter as described by Howe (1920), Taylor (1960) and Littler and Littler (2000).

3.2 Phylogenetic analyses

For the phylogenetic analyses, 17 sequences were generated, 10 sequences of partial LSU with a final alignment of 492 bp, and seven sequences of partial SSU rDNA with a final alignment of 1588 bp. Both markers show the monophyly of *Dictyosphaeria* with high support (Figures 3 and 4), and both markers indicated Siphonocladaceae as not supported (Figures 3 and 4). LSU also provided a better phylogenetic

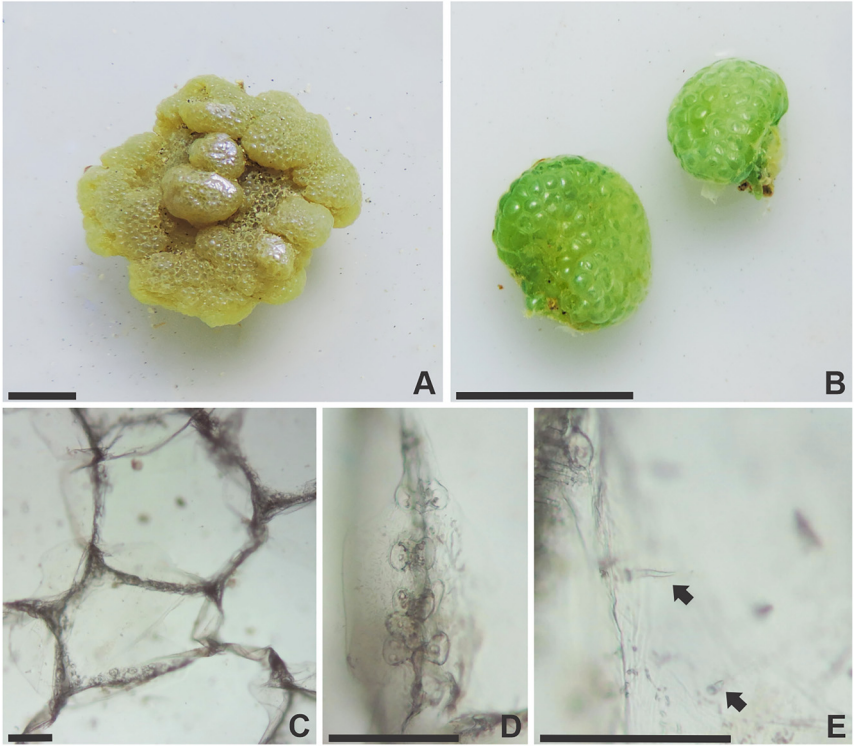

Figure 1: Morphology and anatomy of *Dictyosphaeria versluysii*. (A and B) General view of the sampled individuals. (C) Transverse section showing polygonal cells. (D) Detail of tenacular rounded cells formed between cell surfaces. (E) Cellulose spines (arrows) on internal cell wall. Scale bars: (A and B) = 1 cm; (C) = 200 µm; (D) = 50 µm.

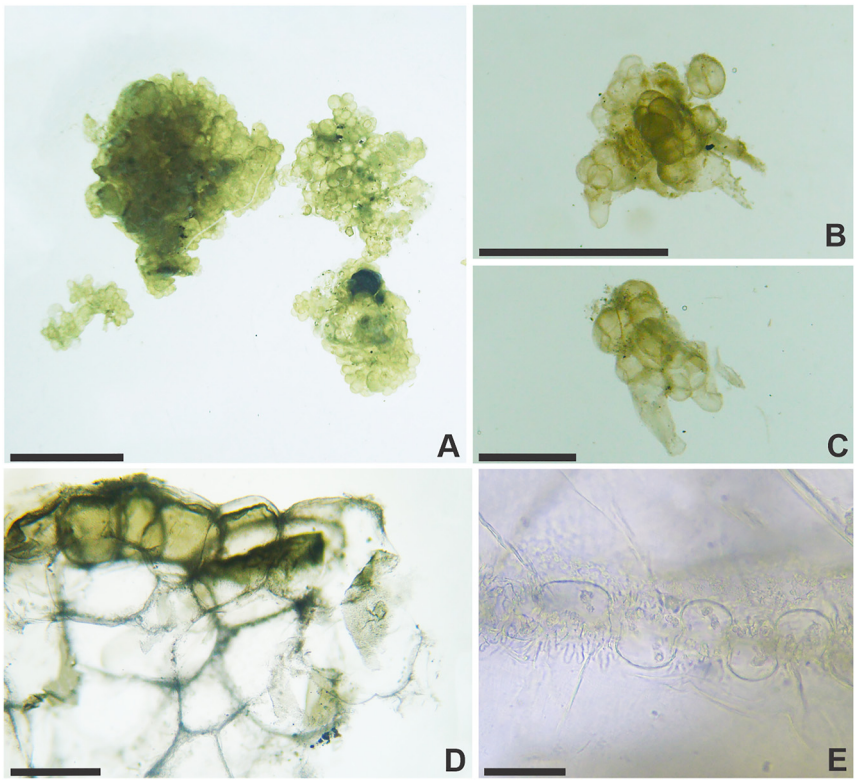

Figure 2: Morphology and anatomy of *Dictyosphaeria ocellata*. (A–C) General view of the sampled individuals. (D) Transverse section showing angular to polyhedral cells. (E) Detail of rounded tenacular cells formed between cell surfaces. Scale bars: (A) = 1 cm; (B and C) = 5 mm; (D) = 750 µm; (E) = 50 µm.

resolution between *Dictyosphaeria* species, and the sequences of *D. cavernosa* and *D. versluysii* were recovered in different clades. Our LSU *D. versluysii* sequences from the Alagoas coast (OK655674), Fernando de Noronha (PP455256), and Trindade Island (PP455254, PP455255) were grouped with high support (BS = 98; PP = 0.99) with *D. versluysii* (MN879584) from an unknown locality, probably Taiwan. Also, while the Japanese *D. cavernosa* (AM503500 and AM503501) sequences formed a highly supported clade, the sequence of *D. cavernosa* from the Seychelles (Indian Ocean) (AM503502) is separated from all other sequences of this species, and its phylogenetic relationship with other sequences was not resolved.

Our newly generated LSU sequences of *D. ocellata* from Trindade Island formed a distinct clade with high support (BS = 98; PP = 0.99). Sequences within *D. ocellata* clade varied by 0–0.2 % (Supplementary Table S3).

The LSU sequences of *D. versluysii* from Alagoas (OK655674) and Trindade (PP455254, PP455255) were identical. Sequences of *D. versluysii* from Brazil were 0.4 % distinct from the sequence of *D. versluysii* (MN879584) probably from Taiwan and 1.7–3.5 % from Tanzania (AM503503), while the distance between *D. verluysii* (AM503503) and *D. cavernosa* (AJ544745), both from Tanzania, was 2.6 %.

In the SSU phylogeny *Dictyosphaeria* sequences grouped with high support (BS = 96; PP = 1) (Figure 4). Three identical Brazilian sequences were identified as *D. versluysii* based on morphology and grouped in a highly supported clade (BS = 99; PP = 0.99). The sequence of *D. cavernosa* from the Seychelles (Indian Ocean) (AM498756) grouped with the Brazilian sequences, but with unresolved relationship, and it diverged from these by 0.5 % (Supplementary Table S4). All sequences of *D. ocellata* from Trindade Island were grouped with high support (BS = 99; PP = 1), and the sequences were identical; the longer branch is due to different sequence lengths.

4 Discussion

The newly generated sequences of *Dictyosphaeria versluysii* and *D. ocellata* from the Brazilian coast and oceanic islands are the first to be obtained in the Atlantic for the genus, providing new insights into *Dictyosphaeria* diversity and distribution. Based on our analyses of both nuclear markers (SSU and LSU), *Dictyosphaeria* is monophyletic; on the other hand, the Siphonocladaceae was not recovered as a monophyletic lineage, in accordance with Leliaert et al. (2003, 2007). The non-monophyly of Siphonocladaceae seems to be aligned with the historical problematic delineation of the family, which is currently an assembly of genera that do not fit in any other family (Leliaert 2004). The LSU and SSU showed similar taxonomic resolution, but the LSU provided a better screen due to its greater variability and the

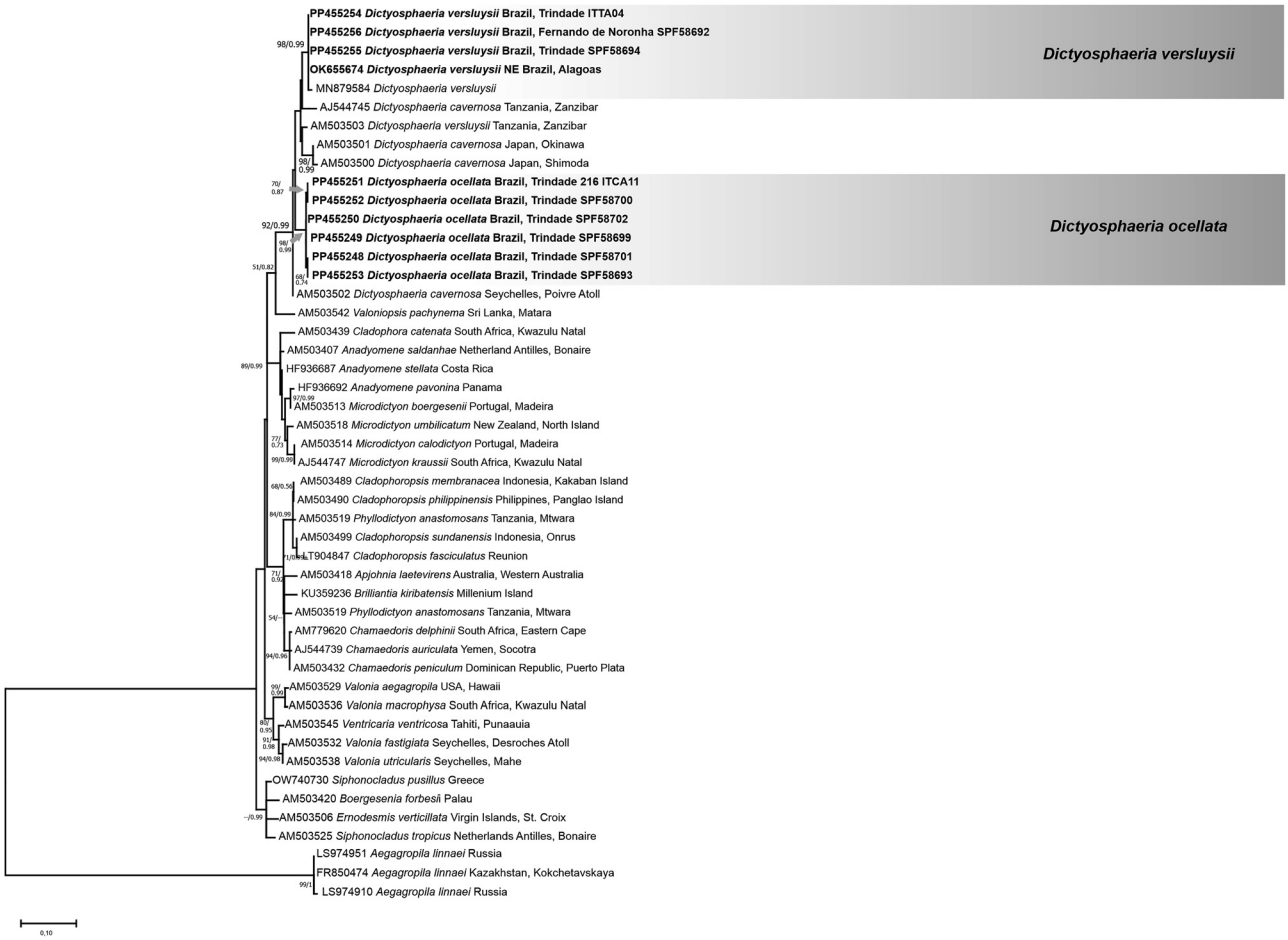

Figure 3: Maximum likelihood of LSU rDNA consensus tree, with 48 sequences in a 492 bp alignment (only values above 70/0.7 for BS/PP are shown in branches). Samples generated in this study are in bold.

greater number of sequences available when compared with SSU.

The Brazilian samples of *D. versluysii* were morphologically in accordance with previous descriptions for this species; however, in small individuals, the inner cell wall spines were absent or rare, mainly in the specimens from both oceanic islands inspected.

Despite the lack of sequences from Malaysia, the type locality of *D. verluysii*, we adopted a conservative approach and considered the specimens from the South Atlantic as *D. verluysii* since they matched the sequence MN879584, probably from Taiwan, a region close to the type locality.

Our sequences of *D. ocellata* are the first of this species worldwide, whose type locality is San Salvador Island (previously Watling's Island) in Bahamas (West Indies). *Dictyosphaeria ocellata* was initially proposed by Howe (1920) as a species of *Valonia*; later Taylor (1960) discussed that young specimens were almost indistinguishable from *Dictyosphaeria*. The transfer of *Valonia ocellata* M. Howe to *Dictyosphaeria* was made by Olsen-Stojkovich (1985). Olsen-Stojkovich et al. (1986) also addressed *D. versluysii*, *D. sericea* Harvey, and *D. ocellata* as *D. versluysii* species complex due to the similarity of the thallus morphology, which could easily generate misidentifications. Our data confirm the phylogenetic placement of *D. ocellata* in the *Dictyosphaeria* genus and show it as a distinct species amongst the others listed for the Caribbean Sea and the southwestern Atlantic Ocean.

The Brazilian material of *D. ocellata* morphologically matches the material included in the original description of the species by Howe (1920), specimen Howe 5585 (Leliaert 2023). This material has a crustose, subspherical thallus with elongated cells, which is very similar to the Brazilian material. However, it differs morphologically from the type specimen designated by Howe (1920), specimen Howe 5090, mainly for not showing hemispherical thalli, but an elongated main axis, mostly filamentous and rarely globose (Leliaert 2023). This difference may represent morphological plasticity related to habitat since the type specimen was found over pneumatophores in a lagoon and the other

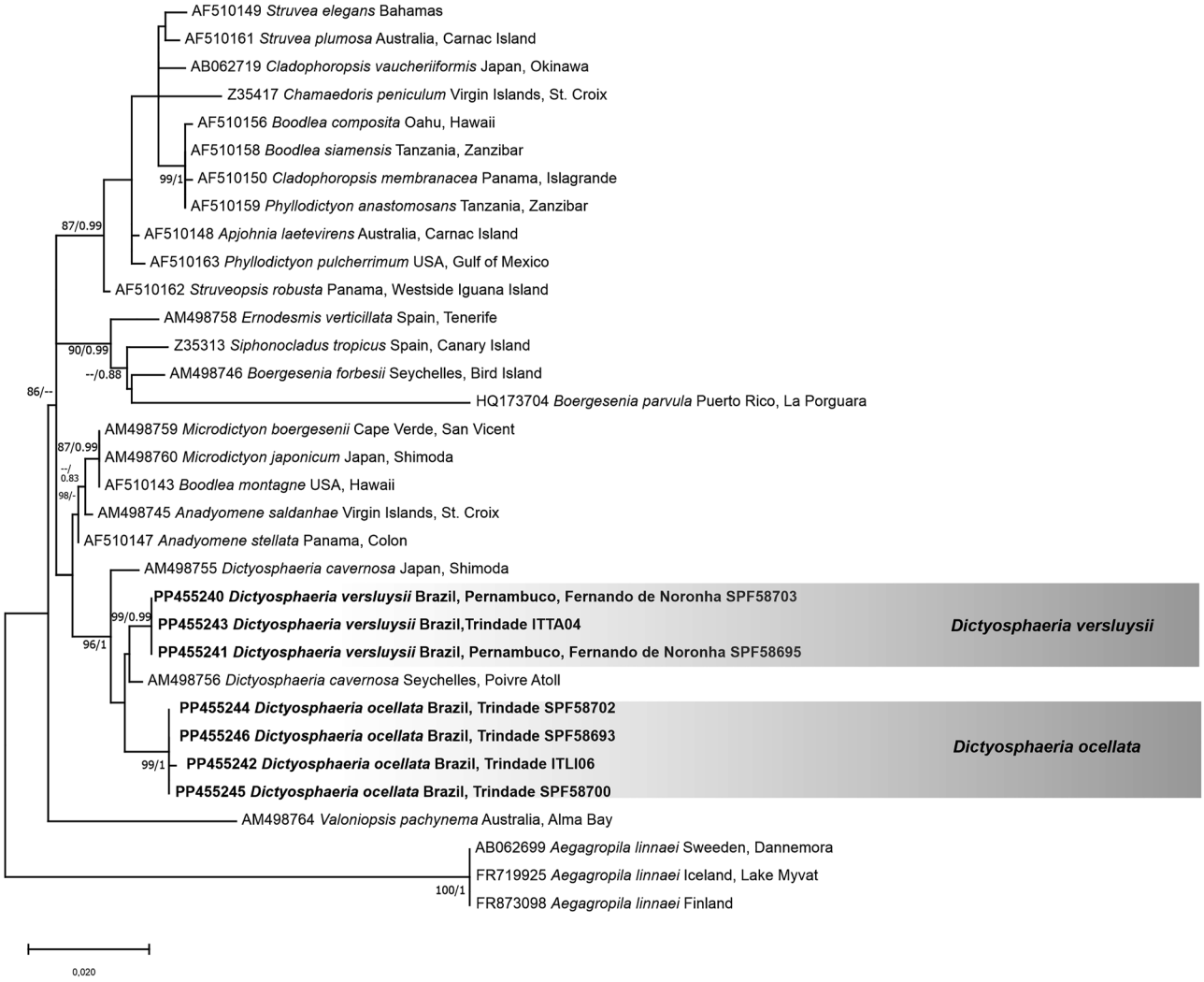

Figure 4: Maximum likelihood of SSU rDNA consensus tree, with 33 sequences in a 1588 bp alignment (only values above 70/0.7 for BS/PP are shown in branches). Samples generated in this study are in bold.

material collected by Howe from the lower intertidal was growing on rocks. On the other hand, it may also indicate a different species, which could only be reliably assigned by sequencing the type material or a topotype with the same morphology. Since this is not possible at present, we prefer to adopt a more conservative approach and consider the original assignment of the species, including this wide morphological range, made by Howe.

Considering the morphology, the *D. ocellata* specimens from the Brazilian islands were significantly smaller than those described by Howe (1920), Taylor (1960), and Littler and Littler (2000). This single difference does not stand as a significant character to separate Trindade specimens from those referred to in the literature and might be related to local ecological responses. The main limitation of phylogenetic studies in *Dictyosphaeria* is the lack of sequences and morphological descriptions associated with them. A broader sampling for this group of siphonocladean algae might help to elucidate its biogeographic patterns, especially because *Dictyosphaeria* is well-distributed in tropical and subtropical areas (Coppejans et al. 2004; Tseng and Chang 1962; Womersley 1984; Yamada 1944). Nonetheless, our phylogenetic data demonstrate that both *D. cavernosa* and *D. versluysii* are species complexes, and both species are widely distributed in the Atlantic, Pacific, and Indian Oceans (Guiry and Guiry 2024). Only the sequencing of the type or type locality material of *D. cavernosa* and *D. versluysii* allied with a wide sampling of both species could reliably resolve the true clades of both taxa.

Since the monophyletic status of the genus seems to be supported, the next step is to investigate species boundaries, through an integrative approach considering their ecology,

evolution, phenotypic plasticity, and distributional data, in addition to including other species of the genus in its phylogenetic reconstruction. Our data reveal the occurrence of one species in the NE Brazilian coast (*D. versluysii*), and two in the oceanic insular ecosystems from the southwestern Atlantic: *D. versluysii* and *D. ocellata*. *Dictyosphaeria ocellata*, until now, occurs only on Trindade Island. The low genetic distance found between populations of *D. versluysii* from the Atlantic (Brazil) and Pacific (probably Taiwan) Oceans indicates differences between populations but is not enough to separate them as distinct species. However, *D. versluysii* from the Indian Ocean (Tanzania) is more divergent indicating the occurrence of cryptic diversity. Wider sampling of this species from different regions, including the type locality, is necessary in order to better understand these questions.

The smaller thallus size of the *D. ocellata* population from Trindade Is. may be related to the insular ecological conditions, but further investigations are needed, since the sequences herein shown are the first for this species, and there is no previous knowledge about *D. ocellata* phenotypic plasticity. Also, the co-occurrence of *D. ocellata* and *D. versluysii* on Trindade Is. highlights the importance of integrative taxonomy, combining morphological and molecular analysis for reliable species identification of *Dictyosphaeria*, especially when analyzing young specimens from the same locality. This first molecular study of *Dictyosphaeria* in Brazil highlights that a broader sampling combined with sequencing of other markers (i.e., ITS) is needed to reveal the diversity of the genus on the Brazilian coast, which is tied to a larger global sampling of the genus, including sequencing of type specimens or topotypes.

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