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Sporolithon franciscanum sp. nov. (Sporolithales, Rhodophyta), a New Rhodolith-Forming Species from Northeast Brazil

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Abstract: This paper describes *Sporolithon franciscanum*, a new rhodolith-forming species of non-geniculate coralline algae found at depths between 47–52 m near the São Francisco river mouth, the second largest and the most extensive drainage basin in Brazil, and also at the Abrolhos Bank, in the world’s largest rhodolith beds. DNA sequences from plastidial *psbA* and *rbcL* markers indicate that the species is unique compared to all other *Sporolithon* species that have thus far been sequenced. Since morpho-anatomical features of the new species are shared with some other *Sporolithon* species, its identification was only confirmed by DNA sequences.

Keywords: coralline algae; molecular phylogeny; morpho-anatomy; *psbA*; *rbcL*; taxonomy

1. Introduction

Coralline red algae (Corallinophycidae, Rhodophyta) have as their main feature the deposition of calcium carbonate polymorphs (calcite, dolomite, magnesite, aragonite, calcite with low magnesium content) on their cell walls [1–4]. Non-geniculate coralline algae are considered one of the most difficult groups within the Rhodophyta in terms of taxonomic identification [5–7]. This perception can be attributed largely to the fact that, unlike most seaweeds, they have a calcified thallus that requires specific and time-consuming laboratory methods for taxonomic analyses [8]. Most identification from order (Corallinales, Hapalidiales, Sporolithales) to species level requires analyses of characteristics related to the reproductive structures (conceptacles or calcified compartments) from the tetra/bisporophytic phases [9]. However, although tetra/bisporophytic phases are the most abundant in nature, many specimens studied only have gametophytic conceptacles or, even more often, have been unfertile (and so excluded from the morpho-anatomical analysis). At the genus and sometimes at species level, the exclusive use of vegetative characteristics may also be useful in identification, such as the shape of epithallial cells or type of cell connections. However, in most cases morpho-anatomical identification in coralline algae is based on a combination of both vegetative and reproductive characteristics [9]. The main problem concerns cryptic species, which cannot be distinguished by morpho-anatomy, but are genetically different species. One example has been seen in the genus *Lithophyllum* Philippi

(Corallinales), in which 13 different species were passed under the name *Lithophyllum stictaeforme* due to their similar morpho-anatomy [10]. On the other hand, a single species/genotype is often represented by different morphologies/phenotypes (e.g., [11]). The incorporation of molecular data into the coralline algae taxonomy has enabled previous classifications to be revised, confirmed or modified, in addition to enabling the identification of cryptic and/or new species established by genetic disparities or not revealed in the phenotype [12–19].

Non-geniculate coralline algae can grow attached to continuous substrates or loosen, forming free-living calcareous nodules named rhodoliths, which, by definition, are composed mainly (>50%) by non-geniculate coralline algae [20,21]. Non-geniculate coralline algae are represented worldwide by at least 75 species known to form rhodoliths [9,15,22–26], and the highest richness of this group (33 taxa) is found in Brazil where the genus *Sporolithon* Heydrich (Sporolithales) holds the largest number of species [26,27].

Sporolithon is characterized by a combination of features, such as: epithallial cells with a trapezoidal ('flared') lumen, contiguous filament cells connected by both cell fusions and secondary pit-connections, and cruciate divided tetrasporangia produced singly within uniporate, calcified compartments, each bearing an apical plug that are borne on a single stalk cell [28,29]. The members of this algal genus are among the main rhodolith-forming algal genera in the southwestern (SW) Atlantic where they are mainly found forming rhodoliths (and then contributing to the formation of rhodolith beds) in mesophotic regions (20–133 m deep) [26,27,30–34], but some species can also be found in shallow (2–7 m) reefs [26,35].

Currently, there are 29 recognized species of *Sporolithon* worldwide [36] from which 12 are recorded in Brazil, namely, *S. amadoi* [26], *S. australasicum* [37], *S. durum* [38], *S. elevatum* [35], *S. episoredion* [35], *S. episporum* [39,40], *S. erythraeum* [37], *S. howei* [37], *S. molle* [31], *S. pacificum* [37], *S. tenue* [32], and *S. yoneshigueae* [33]. With the exception of *S. howei*, which has been recorded to form carbonate concretions, all species were reported to occur in Brazilian rhodolith beds [31–35,37–40]. Among them, only *S. amadoi*, *S. tenue* and *S. yoneshigueae* were assigned based on the analysis of DNA sequences of the type material. The other nine *Sporolithon* species were determined based on specimens identified using only morpho-anatomical analyses. Thus, some of these names may have been incorrectly applied. In addition, and unfortunately, most specimen collections used for this identification were formalin-preserved and DNA sequencing analysis is likely to be unfruitful. Therefore, investments in new samplings of fresh rhodoliths with suitable storage methods for DNA analysis are required to assess the true diversity of these coralline algae in the SW Atlantic. Recently, several molecular studies have reported the existence of cryptic speciation in *Sporolithon*, which led to an increase in the number of species of this genus [15,23–26]. These studies reinforced that comparative analyses of DNA sequences based on sequences of type specimens is the best way to unequivocally identify non-geniculate coralline algae since the morpho-anatomical characteristics alone can be misleading. However, obtaining diagnostic DNA sequences from type material has been a hard task and the main challenge in the current coralline algae taxonomy, since many attempts in doing it have failed [41,42].

There is an ongoing scientific interest in rhodolith beds worldwide due, among other reasons, to the following: (1) The beds are bioengineers and provide a complex three-dimensional matrix (structural complexity) and hard structure that serves as a relatively stable microhabitat for several invertebrates, other macroalgae and fishes, some of which are endemic, rare or commercially important species [10,11,27,43–48]; (2) they are sensitive to the ongoing global climate change (e.g., ocean warming and acidification) that is predicted for the current century [49]; (3) their structures serve as paleoenvironmental indicators of past oceanic conditions [50,51]; (4) rhodolith beds are, alongside coral reefs, one of the main contributors to the global calcium carbonate budget [52–54]. In the tropical southwestern (SW) Atlantic Ocean (Brazil), the rhodolith-forming non-geniculate coralline algae are also known to be the main carbonate builders forming the world's largest rhodolith beds [27] in addition to other extensive carbonate reefs [55,56].

Coralline algae exert species-specific interactions with other key organisms for community structuring. For instance, it has been demonstrated that larval settlement and metamorphosis of some species of coral and other invertebrates occurs preferably or restrictedly in the presence of some specific coralline algae [57–59]. Therefore, knowledge of the rhodolith-forming species is essential for understanding their specific role within the communities. In this context, the aim of this study was to report a new rhodolith-forming *Sporolithon* species that was found near the São Francisco River mouth (the second largest and the most extensive drainage basin within the Brazilian territory [60,61]) and in the Abrolhos Bank (the world's largest rhodolith bed [53]). It is important to note that these two areas are characterized by presenting a high biodiversity associated with rhodoliths [27,62].

2. Materials and Methods

2.1. Sampling

Two specimens were analyzed in this study. The first (RB 797665) was sampled within the scope of the Marseal Project (Universidade Federal de Sergipe and Petrobras) that aims to generate physical, geological, chemical and biological data related to the Sergipe-Alagoas basin (Northeast Brazil). This specimen was collected offshore Aracaju (11°9'49.580" S; 36°53'0.814" W, Sergipe, Brazil, at 47 m deep), during the third campaign of the Marseal Project, in 2011, using a Van Veen grab sediment sampler. The sample was initially preserved in a 70% ethanol solution, and then preserved in silica gel. The second (Voucher GM AF5) was a small, unfertile, rhodolith fragment sampled at the Abrolhos continental shelf (17°54'01" S; 37°54'56" W, Bahia, Brazil, at 52 m deep, G.M. Amado Filho, 11.iii.2012), preserved in silica gel, and with published sequences in GenBank (KP142752), which was used to call *Sporolithon* sp. (voucher GM AF5) in Adey et al. [63] and Richards et al. [15,26]. The first is housed at the Rio de Janeiro Botanical Garden Herbarium (RB) under the voucher RB 797665, while the second is housed at the National Museum of Natural History (NMNH) (US, the United States National Herbarium at NMNH) under the voucher GM AF5. Herbarium abbreviations follow [64], continuously updated.

2.2. Morpho-Anatomical Analyses

Fractures from fertile areas of the holotype were decalcified according to Jesionek et al. [35] and prepared for light microscopy examination using the histological methods described by Maneveldt and Van der Merwe [65]. Other fractures were examined with scanning electron microscopy (SEM) following the protocol used by Bahia et al. [66]. The thallus anatomical terminology followed Chamberlain [67]. The morphological (growth forms) terminology followed Woelkerling et al. [68]. Cell dimensions were measured from all available images as described in Maneveldt et al. [33]. Tetrasporangial compartments and tetrasporangia were measured separately according to Kaewsuralikhit et al. [69]. Since the specimen from Abrolhos (GM AF5) corresponds to an unfertile small fragment, no anatomical analyses were performed for this specimen.

2.3. Molecular Analysis

DNA extraction, amplification and sequencing of the *psbA* genetic marker followed the protocol used by Jesionek et al. [70] and were performed for the specimen from Sergipe (RB 797665). DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit[®] (Qiagen, Crawley, UK) following the modified protocol of Broom et al. [13]. The *psbA* gene was amplified using the primers *psbAF* and *psbAR2* [71]. For the Abrolhos continental shelf specimen (GM AF5), the analysis for *psbA* and *rbcL* followed Adey et al. [63]. The extraction was made following the protocol of Huguey et al. [72], in which after a series of steps, it resulted in a working solution of 10:1 (water:DNA) for PCR and a negative control for each set of extraction. For *psbA* gene amplification, were used the same primers as for the Sergipe specimen (*psbAF* and *psbAR2*) [71], and for *rbcL* two pairs were used (F57—R1150 and F753—RrbcS) [73]. Since all attempts to amplify *rbcL* for the specimen from Sergipe (RB 797665)

failed, it was not possible to use *rbcL* for molecular analysis. The sequences obtained for both markers were assembled and edited in Sequencher v.4.1.4 (Gene Codes Corporation) and edited and aligned in MEGA6 [74] within ClustalW [75,76]. The sequence dataset for the *psbA* analysis was built with the sequence of the holotype generated in this study in addition to the sequence of the paratype from GenBank (KP142752) and 37 others that were downloaded from GenBank from *Sporolithon*, *Heydrichia* and the outgroup (*Lithothamnion glaciale* and *Melyvonnea erubescens*). The sequences dataset for the *rbcL* analysis included the sequence of the paratype (since *rbcL* could not be amplified for the holotype) generated in Adey et al. [63] and 22 others from GenBank from *Sporolithon* and the outgroup (*Heydrichia woelkerlingii* and *Heydrichia cerasina*). The phylogenetic relationships between taxa were inferred in Geneious R7, using Maximum Likelihood (ML) with Randomized Axelerated Maximum Likelihood (RAxML 8) [77] with a bootstrap of 1000 replicates [78] and Bayesian Inference (BI) with Mr. Bayes [79] with four Monte Carlo–Markov chains. Bootstrap and Bayesian posterior probabilities (PP) values were considered high when they were equal to or higher than 70% and/or 0.70, respectively. In this analysis, 5 million generations were launched. The trees were sampled every 1000 generations and 1,250,000 trees were discarded as burn-in. Finally, the standard deviation of the split frequencies was verified in the summary statistics in Geneious R7.

3. Results

The results from *psbA* analyses indicated that the specimen from Sergipe (RB 797665) and the one from the Abrolhos continental shelf (GM AF5) correspond to the same and new species (namely *Sporolithon franciscanum*) (Figure 1). The phylogram shows that *Sporolithon franciscanum* formed a highly supported (93/0.99, ML and BI) clade sister to *S. eltorensis* and an Australian *Sporolithon* sp. (Figure 1). The divergence value between the specimens from Sergipe and Abrolhos continental shelf was 0.1% (an intraspecific value), and the new species diverge from the sister species from the clade by between 6.1–6.5% (Table 1). In *rbcL* analysis, the available sequences positioned two additional species (*Sporolithon dimotum* and a *Sporolithon* sp.) within the *S. franciscanum* clade (Figure 2). The new species diverged from all taxa within its clade by 6–12.3% (Table 1).

Table 1. Pairwise sequence divergence values (%) between *S. franciscanum* and other *Sporolithon* species from the new species phylogenetic clade.

<i>Sporolithon</i> Species	<i>psbA</i>	<i>rbcL</i>
<i>S. franciscanum</i> vs <i>Sporolithon</i> sp. (Australia)	6.5%	6%
<i>S. franciscanum</i> vs <i>S. eltorensis</i>	6.1%	12.3%
<i>S. franciscanum</i> vs <i>S. dimotum</i>	–	6.8%
<i>S. franciscanum</i> vs <i>Sporolithon</i> sp.	–	6.8%

3.1. *Sporolithon franciscanum* L.A.S. Leão & Bahia sp. nov.

Holotype: RB 797665, collected February, 2, 2011, leg. L.C.S. Fontes, J.R. Santos, L.A. Santos, J.B.S. Mendonça, & M.S. Santos, *psbA* - GenBank MT214482.

Paratype: GMAF5, collected March, 11, 2012, leg. GM Amado Filho, *psbA* - GenBank KP142752, *rbcL* - GenBank KP142786 (Table S1).

Type locality: Aracaju, Sergipe State, Brazil (11°9′49.580″ S, 36°53′0.814″ W), subtidal region (47 m deep), free-living form as rhodolith.

Etymology: The specific epithet *franciscanum* is derived from the main river of Sergipe-Alagoas continental shelf, the São Francisco River.

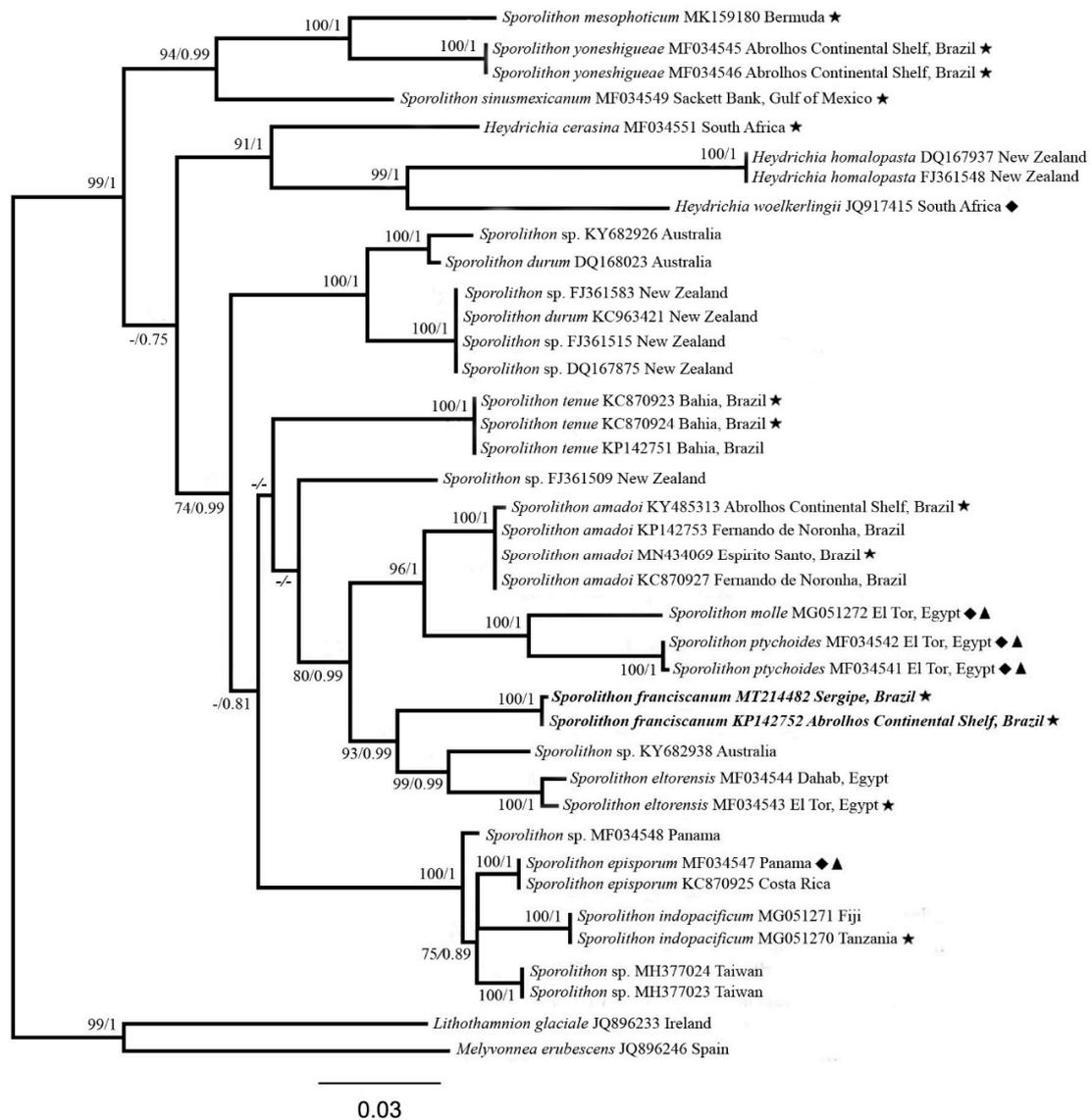


Figure 1. Phylogenetic tree inferred from RAXML (Randomized Axelerated Maximum Likelihood) and BI (Bayesian inference) analyses of the *psbA* dataset. Bootstrap support (1000 replicates) and Bayesian posterior probabilities (PP) are indicated at nodes. Bootstrap values lower than 70% and PP lower than 0.70 are not shown. *Melyvonnea erubescens* and *Lithothamnion glaciale* were used as outgroups. *Sporolithon franciscanum* sp. nov. is marked in boldface type. Stars represent holotype, isotype, neotype, or paratype specimens; diamonds represent topotype specimens (specimens from the type locality); triangles represent species whose identification was confirmed by comparison of DNA sequences with type material.

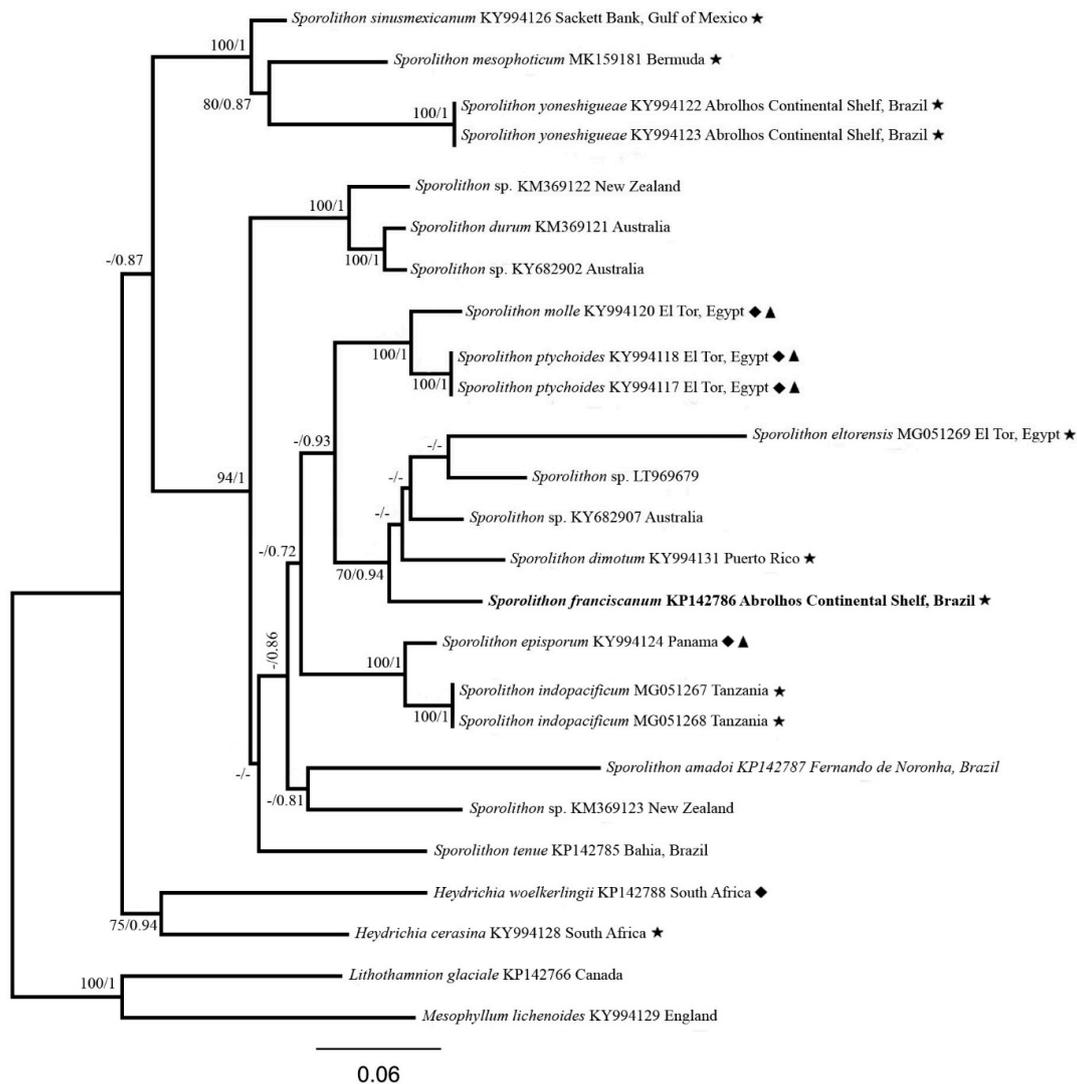


Figure 2. Phylogenetic tree inferred from RAxML and BI analyses of the *rbcL* dataset. Bootstrap support (1000 replicates) and Bayesian posterior probabilities (PP) are indicated at nodes. Bootstrap values lower than 70% and PP lower than 0.70 are not shown. *Lithothamnion glaciale* and *Mesophyllum lichenoides* were used as outgroups. *Sporolithon franciscanum* sp. nov. is marked in boldface type. Stars represent holotype, isotype, neotype, or paratype specimens; diamonds represent topotype specimens (specimens from the type locality); triangles represent species whose identification was confirmed by comparison of DNA sequences with type material.

3.2. Habit and Vegetative Anatomy

The external morphology and anatomy of two specimens were analyzed: A tetrasporophyte holotype and an unfertile paratype. The holotype was found composing the surface of a multispecific rhodolith measuring 4–7 cm in diameter (Figure 3A). The paratype constitutes a small rhodolith fragment (Figure 3B). Thalli were encrusting, with smooth surface, and pink to purple color (Figure 3A).

Thallus dorsiventrally organized, monomerous, 500–1000 μm thick, with more than 20 cell layers (Figure 3C). Medulla plumose (non-coaxial) (Figure 3C), comprise 8–17 filaments aligned in more or less parallel mode to the substratum. Medullary filaments comprise square to elongated cells, measuring 7–22 μm in length and 4–10 μm in diameter. Cortical filaments comprise square to elongated cells, measuring 6–19 μm in length and 3–8 μm in diameter. Contiguous cortical filaments are joined by both secondary pit connections and cell fusions (Figure 3D); cell fusions predominate by a ratio of 2:1. Subepithallial initials square to rectangular, with 6–11 μm in length and 4–7 μm in diameter, as long as

or longer than subtending cells (Figure 3E). Epithallial cells single layered, flattened with flared upper corners (Figure 3E), and measuring 2–5 μm in length and 3–7 μm in diameter. Trichocytes absent.

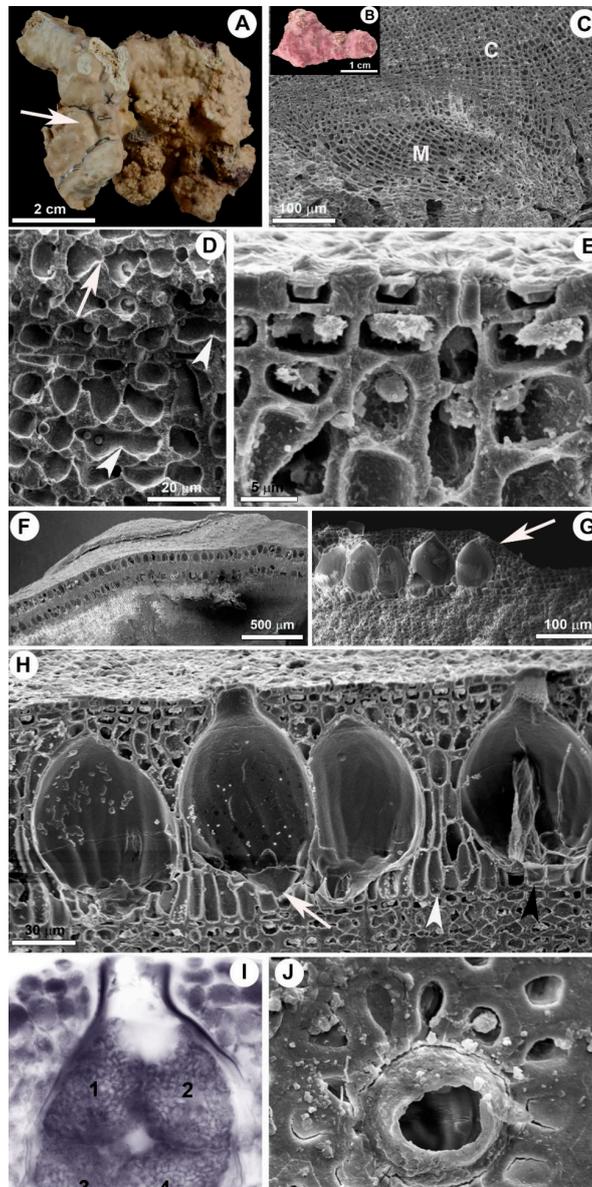


Figure 3. *Sporolithon franciscanum* sp. nov. holotype (RB 797665) and paratype (GM AF5). (A) Habit of holotype; scale bar = 2 cm. (B) Habit of paratype; scale bar = 1 cm. (C) Scanning electron micrograph (SEM) of a vertical section of the thallus showing the monomerous, plumose (non-coaxial) construction, with medullary (M) and cortical (C) filaments; scale bar = 100 μm . (D) SEM of a vertical section showing a secondary pit connection (arrow) and a cell fusion (arrowhead) in the same thallus; scale bar = 20 μm . (E) SEM view of the upper portion of the thallus showing flared epithallial and square to rectangular subepithallial cells; scale bar = 5 μm . (F) SEM view of the thallus showing tetrasporangial compartments buried in a distinct layer; scale bar = 500 μm . (G) SEM view of the upper portion of the thallus showing tetrasporangial chambers raised, 3–5 cells (including epithallial cells), above the surrounding thallus surface; scale bar = 100 μm . (H) SEM view of the upper portion of the thallus showing the paraphyses (white arrowhead) between tetrasporangial chambers, a tetrasporangia subtended by a single stalk cell (arrow) and chambers with a basal layer of elongated cells (black arrowhead); scale bar = 30 μm . (I) Vertical section showing a cruciately divided tetrasporangia (1–4); scale bar = 15 μm . (J) SEM view of a tetrasporangial chamber pore and the surrounding rosette cells; scale bar = 10 μm .

3.3. Reproductive Anatomy

Tetrasporangial chambers clustered into raised sori, 3–5 cells (including epithelial cell), above the surrounding thallus surface (Figure 3G). Individual tetrasporangial chambers uniporate, elliptical, measuring 30–60 µm in diameter and 70–100 µm high, clustered in large groups, separated by 0–5 paraphyses (mostly 0–1) comprise 3–4 elongate cells each (Figure 3H). Chambers possess a basal layer of elongated cells (Figure 3H). In surface view, chamber pores possess 10–16 µm in diameter and are surrounded by 11–13 rosette cells (Figure 3J). Entire tetrasporangial sori become buried within the thallus (Figure 3F). Each chamber bears only one tetrasporangium measuring 60–80 µm in length and 30–56 µm in diameter (Figure 3I). Tetrasporangia are cruciately divided (Figure 3I). Tetrasporangia bear an apical pore plug and are borne on a single triangular stalk cell (Figure 3H).

3.4. Diagnosis

The diagnostic features of the new species are as follows: *psbA* DNA sequence from the holotype (MT214482) and the sequences from the paratype: *psbA* (KP142752) and *rbcl* (KP142786). No morpho-anatomical diagnostic features were found to distinguish this new species from other known *Sporolithon* species.

4. Discussion

The divergence values for both markers (*psbA* and *rbcl*) (Table 1) between *Sporolithon franciscanum* sp. nov. and the species that lie within its phylogenetic clade, are greater than those between other closely related species in *Sporolithon* (e.g., in *psbA* analysis, *S. episporum* and *S. indopacificum* diverge by 2.7% [25]; and in *rbcl* analysis, the divergence value between *S. molle* and *S. ptychoides* is 3.3% [15]). These results confirm the uniqueness of the new species.

Sporolithon franciscanum, the nearby clade of the species *S. ptychoides* and *S. molle*, and the sister species in *rbcl* phylogram, *S. dimotum*, cannot be distinguished morpho-anatomically, as they have tetrasporangial chambers that are overgrown and buried, tetrasporangial chambers with a basal layer of elongated cells and a similar number of rosette cells that surround each tetrasporangial chamber pore (Table 2). *Sporolithon franciscanum* is also morpho-anatomically similar to its sister species, *S. eltorensis*, in the *psbA* phylogram except for the abundance of secondary pit connections versus cell fusions; in the new species, cell fusions predominate at a rate of 2:1, and in *S. eltorensis*, this ratio is 1:1 [15]. However, a larger sample size is needed to confirm whether the ratios of secondary pit connections to cell fusions are truly a useful characteristic separating these species.

Table 2. Characteristics found to be informative in separating species of *Sporolithon* occurring in the Atlantic Ocean for which recent detailed descriptions are available. Descriptions are included only for species for where type/'topotype' specimens and for where representative specimens were confirmed by DNA sequence data. ND = No data provided. * Measure taken from Richards et al. [15]. ** Based on images from field-collected specimens provided by Richards et al. [24]. *** Measure taken from Keats et al. [80].

References and Algal Characteristics	Species						
	<i>S. episorum</i>	<i>S. franciscanum</i>	<i>S. mesophoticum</i>	<i>S. amadoi</i>	<i>S. sinuxmexicanum</i>	<i>S. tenue</i>	<i>S. yoneshigueae</i>
References	Keats & Chamberlain [80]	This study	Richards et al. [15]	Richards et al. [26]	Richards & Fredericq [24]	Bahia et al. [32]	Bahia et al. [33]
Locality	Point Toro, near Colon, Canal Zone (Panama)	Southwestern Atlantic (Aracaju, Sergipe, and Northeast region (Brazil))	Plantagenet (Argus) Bank (Southwest of Bermuda)	Recifes Esquecidos, (Espirito Santo, Brazil)	Sackett Bank, NWGMx (USA)	Southwestern Atlantic (Brazil)	Abrolhos continental shelf (Bahia, Brazil)
Growth form	Flat to lumpy	Encrusting	Encrusting	Encrusting to Warty to lumpy	Encrusting	Encrusting to warty to fruticose	Encrusting to warty to lumpy to fruticose
Habit	Epilithic or free living as rhodolith	Epilithic or free living as rhodolith	Epilithic, epizoic, or free living as rhodolith	Epilithic, epizoic, or free living as rhodolith	Free living as rhodolith	Free living as rhodolith	Free living as rhodolith
Relative abundance of secondary pit connections versus cell fusions (proportion)	Primarily by secondary pit connections; cell fusions rare	Cell fusions predominate by a ratio of 2:1	Cell fusions (Secondary pit-connections not observed)	Secondary pit connections predominate by a ratio of 2:1;	Abundant cell fusions (secondary pit connections not observed)	Secondary pit connections predominate at a ratio of 2–3:1	Cell fusions predominate by a ratio of 2–3:1
Vegetative thallus thickness	ND	>250 µm (500–1000 µm)	120–150 µm	ND (>20 cell layers)	>250 µm (at least 800 µm) **	45–250 µm (less than 20 cell layers)	>250 µm (1000–2500 µm)
Tetrasporangia length	up to 50 µm	60–90 µm	ND	ND	ND	50–75 µm	90–140 µm
Tetrasporangia diameter	ca. 30 µm	30–60 µm	ND	ND	ND	30–40 µm	80–100 µm
Tetrasporangial chamber length	50–70 µm	70–100 µm	ND	65–108 µm	83 µm	70–95 µm	140–210 µm
Tetrasporangial chamber diameter	25–40 µm	30–60 µm	ND	41–64 µm	59 µm	35–55 µm	70–130 µm
Tetrasporangial chamber pore diameter	up to 30 µm	10–16 µm	16–23 µm*	8.5–16.5 µm	16–22 µm **	9–14 µm	35–43 µm
Number of rosette cells surrounding tetrasporangial chamber pore	12–16 ***	11–13	10–14	9–13	10–12	8–12	19–24
Layer of elongated cells at the base of tetrasporangial chamber	Present	Present	ND	Present	Absent	Absent	Present
Buried tetrasporangial chambers	Absent	Present	Absent	Present	Absent	Absent	Present
Position of tetrasporangial chambers relative to the surrounding vegetative surface (number of cells)	Slightly raised (3–4 cells)	Raised, 3–5 cells (incl. epithallial cell)	Flush to slightly raised (ND for number of cells layers)	Raised (ND for number of cells layers)	Slightly raised (ND for number of cells layers) **	Raised (5–7 cells)	Raised 5–8 cells

Considering *Sporolithon* species that occur in the SW Atlantic (*S. amadoi*, *S. episporum*, *S. tenue* and *S. yoneshigueae*), the analysis has shown that they are all positioned in distinct phylogenetic clades from that of *S. franciscanum*. However, it has been demonstrated that the new species and *Sporolithon amadoi* cannot be distinguished morpho-anatomically. Brazilian specimens with the same features present in *S. franciscanum* and *S. amadoi* (confirmed to occur in Southeast and Northeast Brazil in addition to the Gulf of Mexico) have been erroneously determined as the single species *S. ptychooides* [26,30,81] and considered one of the most common rhodolith-forming species in the SW Atlantic [27,81]. This finding suggests that *S. franciscanum* could be more common and have a wider distribution in the SW Atlantic; this result can only be confirmed through a review of all specimens assigned to *S. ptychooides* for Brazil, including DNA analysis and likely requiring new, freshly collected material. On the other hand, the following features were found to be useful to separate *S. franciscanum* from the other remaining SW Atlantic *Sporolithon* species: Thallus thickness, relative abundance of secondary pit connections and cell fusions, presence/absence of buried tetrasporangial chambers, dimensions of tetrasporangial chambers, number of rosette cells surrounding tetrasporangial pore and tetrasporangial chamber pore diameter (Table 2).

Richards et al. [15] concluded that the genus *Sporolithon* has undergone extensive speciation processes with little concomitant phenotypical/morpho-anatomical modifications. Indeed, there are records of fossil *Sporolithon* specimens dated from over 10 million years and attributed to present-day species due to anatomical similarity (e.g., *S. ptychooides* from the middle Miocene from Romania) [82]. Therefore, a global effort to review *Sporolithon* species including DNA sequencing is necessary to assess the real richness of this genus and understand its role within the past and present carbonate ecosystems.

Rhodolith beds are subjected to multiple threats as direct and indirect consequences of human activities, such as extraction for commercial use of calcium carbonate, bottom fishing trawling, oil and gas activities [49]. Oil and gas production can be considered the main concern to the rhodolith beds inhabiting the Sergipe-Alagoas continental shelf [62,83,84] by promoting massive sediment dislodgment, resulting mainly in burial by sediment deposition, and hampering photosynthesis of coralline algae by light attenuation as a consequence of sediment suspension [85]. In this context, taxonomic inventories are becoming even more urgent to support local conservation and management actions directed towards rhodolith beds.

5. Conclusions

This study demonstrated that the genus *Sporolithon* is richer than previously thought revealing a new cryptic species to science morpho-anatomically similar to other Atlantic species. This finding corroborates results from previous studies indicating that comparative analyses of DNA sequences based on sequences of type specimens is required to unequivocally identify *Sporolithon* species. Based on these observations, we find ourselves having to question all reports, based only on morpho-anatomy of the *Sporolithon* species in SW Atlantic, particularly of *S. australasicum*, *S. durum*, *S. elevatum*, *S. episoredion*, *S. episporum*, *S. erythraeum*, *S. howei*, and *S. molle*. Considering the ecological significance of *Sporolithon* as an important rhodolith-forming genus and that coralline algae are among the most sensitive calcified organisms to the emergent threat of ocean acidification (with negative effects that could be amplified by global warming), taxonomic studies like the present one are becoming even more urgent to support local conservation and management actions directed towards rhodolith beds.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/12/5/199/s1>, Table S1: List of specimens used in the phylogenetic analyses, including specimen identification number, locality, collector names, collection date and GenBank accession number.

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References

1. Silva, P.C.; Johansen, H.W. A reappraisal of the order Corallinales (Rhodophyceae). *Br. Phycol. J.* **1986**, *21*, 245–254. [[CrossRef](#)]
2. Borowitzka, M.A. Calcification in algae: Mechanisms and the role of metabolism. *Crit. Rev. Plant Sci.* **1987**, *6*, 1–45. [[CrossRef](#)]
3. Nash, M.C.; Troitzsch, U.; Opdyke, B.N.; Trafford, J.M.; Russel, B.D.; Kline, D.I. First discovery of dolomite and magnesite in living coralline algae and its geobiological implications. *Biogeosciences* **2011**, *8*, 3331–3340. [[CrossRef](#)]
4. Kraysky-Self, S.; Richards, J.L.; Rahmatian, M.; Fredericq, S. Aragonite infill in overgrown conceptacles of coralline Lithothamnion spp. (hapalidiaceae, hapalidiales, rhodophyta): New Insights in biomineralization and phylomineralogy. *J. Phycol.* **2016**, *52*, 161–173. [[CrossRef](#)] [[PubMed](#)]
5. Woelkerling, W.M.J. *The Coralline Red Algae: An Analysis of the Genera and Sub-Families of Nongeniculate Corallinaceae*; British Museum Natural History and Oxford University Press: London, UK, 1988; p. 280.
6. Irvine, L.M.; Chamberlain, Y.M. *Seaweeds of the British Isles. Volume I Rhodophyta. Part 2B Corallinales, Hildenbrandiales*; Her Majesty's Stationary Office: London, UK, 1994; p. 276.
7. Woelkerling, W.J.; Lamy, D. *Non-Geniculate Coralline Red Algae and the Paris Museum: Systematics and Scientific History*; Publications Scientifiques du Muséum/ADAC: Paris, France, 1998; pp. 1–767.
8. Maneveldt, G.; Chamberlain, Y.M.; Keats, D.W. A catalogue with keys to the non-geniculate coralline algae (Corallinales, Rhodophyta) of South Africa. *S. Afr. J. Bot.* **2008**, *555*–566. [[CrossRef](#)]
9. Harvey, A.S.; Woelkerling, W.J. A guide to nongeniculate coralline red algal (Corallinales, Rhodophyta) rhodolith identification. *Cienc. Mar.* **2007**, *33*, 411–426. [[CrossRef](#)]
10. Amado-Filho, G.M.; Maneveldt, G.; Pereira-Filho, G.H.; Manso, R.C.; Bahia, R.G.; Barreto, M.B.B.; Guimaraes, S.M.P.B. Seaweed diversity associated with a Brazilian tropical rhodolith bed. *Cienc. Mar.* **2010**, *36*, 371–391.
11. Pereira-Filho, G.H.; Amado Filho, G.M.; Guimarães, S.M.P.B.; Moura, R.L.; Sumida, P.Y.G.; Abrantes, D.P.; Bahia, R.G.; Guth, A.Z.; Jorge, R.; Francini-Filho, R.B. Reef Fish and Benthic assemblages of the Trindade and Martin Vaz island group, Southwestern Atlantic. *Braz. J. Oceanogr.* **2011**, *59*, 201–212. [[CrossRef](#)]
12. Bailey, J.C.; Chapman, R.L. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) determined by 18S rDNA gene sequence analysis. In *Cytology, Genetics and Molecular Biology of Algae*; Chaudhary, B.R., Agrawal, S.B., Eds.; SPB Academic Publishing: Amsterdam, The Netherlands, 1996; pp. 363–376.
13. Broom, J.E.S.; Hart, D.R.; Farr, T.J.; Nelson, W.A.; Neill, K.F.; Harvey, A.S.; Woelkerling, W.J. Utility of psbA and nSSU for phylogenetics reconstruction in the Corallinales based on New Zealand taxa. *Mol. Phylogenet. Evol.* **2008**, *46*, 958–973. [[CrossRef](#)]
14. Nelson, W.A.; Sutherland, J.E.; Farr, T.J.; Hart, D.R.; Neill, K.F.; Kim, H.J.; Yoon, H.S. Multi-gene phylogenetic analyses of New Zealand coralline algae: *Corallinapetra novaezelandiae* gen. et and recognition of the Hapalidiales ord. nov. *J. Phycol.* **2015**, *51*, 454–468. [[CrossRef](#)]
15. Richards, J.L.; Sauvage, T.; Schmidt, W.E.; Fredericq, S.; Huguey, J.R.; Gabrielson, P. The coralline genera *Sporolithon* and *Heydrichia* (Sporolithales, Rhodophyta) clarified by sequencing type material of their generitypes and other species. *J. Phycol.* **2017**, *53*, 1044–1059. [[CrossRef](#)] [[PubMed](#)]
16. Hind, K.R.; Gabrielson, P.W.; Saunders, G.W. Molecular-assisted alpha taxonomy reveals pseudocryptic diversity among species of Bossiella (Corallinales, Rhodophyta) in the eastern Pacific Ocean. *Phycologia* **2014**, *53*, 443–456. [[CrossRef](#)]
17. Caragnano, A.; Foetisch, A.; Maneveldt, G.; Millet, L.; Liu, L.C.; Lin, S.M.; Rodondi, G.; Payri, C.E. Revision of Corallinaceae (Corallinales, Rhodophyta): Recognizing Dawsoniolithon, gen. nov., Parvicellularium gen. nov. and Chamberlainioideae subfam. nov. containing Chamberlainium gen. nov. and Pneophyllum. *J. Phycol.* **2018**, *54*, 391–409. [[CrossRef](#)] [[PubMed](#)]
18. Liu, L.C.; Lin, S.M.; Caragnano, A.; Payri, C. Species diversity and molecular phylogeny of non-geniculate coralline algae (Corallinophycidae, Rhodophyta) from Taoyuan algal reefs in northern Taiwan, including Crustaphytum gen. nov. and three new species. *J. Appl. Phycol.* **2018**, *30*, 3455–3469. [[CrossRef](#)]

19. Aki, K.; Masasuke, B. Distribution of *Lithophyllum kuroshioense* sp. nov., *Lithophyllum subtile* and *L. kaiseri* (Corallinales, Rhodophyta), but not *L. kotschyianum*, in the northwestern Pacific Ocean. *Phycologia* **2019**. [CrossRef]
20. Steneck, R.S. The ecology of coralline algal crusts: Convergent patterns and adaptative strategies. *Annu. Rev. Ecol. Systemat.* **1986**, *17*, 273–303. [CrossRef]
21. Foster, M.S. Rhodoliths: Between rocks and soft places—Minireview. *J. Phycol.* **2001**, *37*, 659–667. [CrossRef]
22. Foster, M.S.; Amado-Filho, G.M.; Kamenos, N.A.; Riosmena-Rodríguez, R.; Steller, D.L. Rhodoliths and Rhodoliths beds. In *Research and Discoveries: The Revolution of Science through SCUBA*; Lang, M.A., Marinelli, R.L., Roberts, S.J., Taylor, P.R., Eds.; Smithsonian Institution Scholarly Press: Washington, DC, USA, 2013; pp. 143–155.
23. Maneveldt, G.W.; Gabrielson, P.; Kangwe, J. *Sporolithon indopacificum* sp. nov. (Sporolithales, Rhodophyta) from tropical western Indian and western Pacific oceans: First report, confirmed by DNA sequence data, of a widely distributed species of *Sporolithon*. *Phytotaxa* **2017**, *326*, 115–128. [CrossRef]
24. Richards, J.L.; Fredericq, S. *Sporolithon sinuamexicanum* sp. nov. (Sporolithales, Rhodophyta): A new rhodolith-forming species from deepwater rhodolith beds in the Gulf of Mexico. *Phytotaxa* **2018**, *350*, 135–146. [CrossRef]
25. Richards, J.L.; Gabrielson, P.; Schneider, C.W. *Sporolithon mesophoticum* sp. nov. (Sporolithales, Rhodophyta) from Plantagenet Bank off Bermuda at a depth of 178 m. *Phytotaxa* **2018**, *385*, 67–76. [CrossRef]
26. Richards, J.L.; Bahia, R.G.; Jesionek, M.B.; Fredericq, S. *Sporolithon amadoi* sp. nov. (Sporolithales, Rhodophyta), a new rhodolith-forming non-geniculate coralline alga from offshore the northwestern Gulf of Mexico and Brazil. *Phytotaxa* **2019**, *423*, 49–67. [CrossRef]
27. Amado-Filho, G.M.; Bahia, R.G.; Pereira-Filho, G.H.; Longo, L.L. South Atlantic Rhodolith Beds: Latitudinal distribution, species composition, structure and ecosystem functions, threats and conservation status. In *Rhodolith/Maerl Beds: A Global Perspective*; Riosmena-Rodrigues, R., Nelson, W., Aguirre, J., Eds.; Springer: New York, NY, USA, 2017; Volume 15, pp. 229–318.
28. Verheij, E. The genus *Sporolithon* (Sporolithaceae fam. nov., Corallinales, Rhodophyta) from the Spermonde Archipelago, Indonesia. *Phycologia* **1993**, *32*, 184–196. [CrossRef]
29. Le Gall, L.; Payri, C.E.; Bittner, L.; Saunders, G.W. Multigene phylogenetic analyses support recognition of the *Sporolithales* ord. nov. *Mol. Phylogenet. Evol.* **2010**, *54*, 302–305. [CrossRef] [PubMed]
30. Bahia, R.G.; Riosmena-Rodríguez, R.; Maneveldt, G.W.; Amado-Filho, G.M. First report of *Sporolithon ptychooides* (Sporolithales, Corallinophycidae, Rhodophyta) for the Atlantic Ocean. *Phycol. Res.* **2011**, *59*, 64–69. [CrossRef]
31. Bahia, R.G.; Amado-Filho, G.M.; Maneveldt, G.W. *Sporolithon molle* (Heydrich) Heydrich (Sporolithales, Corallinophycidae, Rhodophyta): An addition to the Atlantic flora found on a remote oceanic island. *Cryptogam. Algal.* **2014**, *35*, 7–14. [CrossRef]
32. Bahia, R.G.; Amado-Filho, G.M.; Maneveldt, G.W.; Adey, W.H.; Johnson, G.; Marins, B.V.; Longo, L.L. *Sporolithon tenue* sp. nov. (Sporolithales, Corallinophycidae, Rhodophyta): A new rhodolith-forming species from the tropical southwestern Atlantic. *Phycol. Res.* **2014**, *62*, 44–54. [CrossRef]
33. Bahia, R.G.; Amado-Filho, G.M.; Maneveldt, G.W.; Adey, W.H.; Johnson, G.; Jesionek, M.B.; Longo, L.L. *Sporolithon yoneshigueae* sp. nov. (Sporolithales, Corallinophycidae, Rhodophyta), a new rhodolith-forming coralline alga from the southwest Atlantic. *Phytotaxa* **2015**, *224*, 140–158. [CrossRef]
34. Henriques, M.C.; Coutinho, L.M.; Riosmena-Rodríguez, R.; Barros-Barreto, M.B.; Khader, S.; Figueiredo, M.A. Three deep water species of *Sporolithon* (Sporolithales, Rhodophyta) from the Brazilian continental shelf, with the description of *Sporolithon elevatum* sp. nov. *Phytotaxa* **2014**, *190*, 320–330. [CrossRef]
35. Jesionek, M.B.; Bahia, R.G.; Hernández-Kantún, J.; Adey, W.H.; Yoneshigue-Valentin, Y.; Longo, L.L.; Amado-Filho, G.M. A taxonomic account of non-geniculate coralline algae (Corallinophycidae, Rhodophyta) from shallow reefs of the Abrolhos Bank, Brazil. *Algae* **2016**, *31*, 317–340. [CrossRef]
36. Guiry, M.D.; Guiry, G.M. AlgaeBase. World-Wide Electronic Publication. National University of Ireland, Galway. Available online: <http://www.algaebase.org> (accessed on 26 April 2020).
37. Tomita, N.Y. Contribuição ao conhecimento do gênero *Sporolithon* (Corallinaceae, Cryptonemiales) no Brasil. Ph.D. Thesis, Universidade de São Paulo, São Paulo, Brazil, 1976; p. 138.
38. Wynne, M.J. *A checklist of benthic marine algae of the tropical and subtropical Western Atlantic: Third revision*; J. Cramer in der Gebr. Borntraeger Verlagsbuchhandlung: Stuttgart, Germany, 2011; p. 166.

39. Costa, I.O.; Horta, P.A.; Bergstrom, E.R.; Nunes, J.M.C. Taxonomic study of crustose coralline algae off the northeastern Brazilian coast. *Phytotaxa* **2014**, *190*, 130–161. [[CrossRef](#)]
40. Bahia, R.G.; Maneveldt, G.W.; Amado-Filho, G.M.; Yoneshigue-Valentin, Y. New diagnostic characters for the order Sporolithales (Corallinophycidae, Rhodophyta). *J. Phycol.* **2015**, *51*, 1137–1146. [[CrossRef](#)] [[PubMed](#)]
41. Rosler, A.; Perfectti, F.; Peña, V.; Braga, J. Phylogenetic relationships of Corallinaceae (Corallinales, Rhodophyta): Taxonomic implications for reef-building corallines. *J. Phycol.* **2016**, *52*, 412–431. [[CrossRef](#)] [[PubMed](#)]
42. Pezzolesi, L.; Peña, V.; Le Gall, L.; Gabrielson, P.W.; Kaleb, S.; Huguey, J.R.; Rodondi, G.; Hernandez-Kantún, J.J.; Falace, A.; Basso, D.; et al. Mediterranean lithophyllum stictiforme (corallinales, rhodophyta) is a Genetically diverse species complex: Implications for species circumscription, biogeography and conservation of coralligenous habitats. *J. Phycol.* **2019**, *55*, 473–492. [[CrossRef](#)] [[PubMed](#)]
43. Hall-Spencer, J.M.; Grall, J.; Moore, P.G.; Atkinson, R.J.A. Bivalve fishing and maerl-bed conservation in France and the UK—Retrospect and prospect. *Aquat. Conserv.* **2003**, *13*, S33–S41. [[CrossRef](#)]
44. Amado-Filho, G.M.; Maneveldt, G.; Manso, R.C.C.; Marins-Rosa, B.V.; Pacheco, M.R.; Guimarães, S.M.P.B. Structure of rhodolith beds from 4 to 55 meters deep along the southern coast of Espírito Santo State, Brazil. *Cienc. Mar.* **2007**, *33*, 399–410. [[CrossRef](#)]
45. Nelson, W.A.; Neill, K.; Farr, T.; Barr, N.; D’Archino, R.; Miller, S.; Stewart, R. Rhodolith Beds in Northern New Zealand: Characterisation of Associated Biodiversity and Vulnerability to Environmental Stressors. *N. Z. Aquat. Environ. Biodivers. Rep.* **2012**, *99*, 102.
46. Foster, M.S.; Riosmena-Rodríguez, R.; Steller, D.; Woelkerling, W.M.J. Living rhodolith beds in the Gulf of California and their significance for paleoenvironmental interpretation. In *Pliocene Carbonates and Related Facies Flanking the Gulf of California, Baja California, Mexico*; Special Paper Number 31; Johnson, M.E., Ledesma-Vazquez, J., Eds.; Geological Society of America: Boulder, CO, USA, 1997; pp. 127–139.
47. Steller, D.L.; Riosmena-Rodríguez, R.; Foster, M.S.; Roberts, C. Rhodolith bed diversity in the Gulf of California: The importance of rhodolith structure and consequences of anthropogenic disturbances. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2003**, *13*, 5–20. [[CrossRef](#)]
48. Littler, M.M.; Littler, D.S. Coralline algal rhodoliths form extensive benthic communities in the gulf of Chiriqui, Pacific Panama. *Coral Reefs* **2008**, *27*, 553. [[CrossRef](#)]
49. Martin, S.; Hall-Spencer, J.M. Effects of Ocean Warming and Acidification on Rhodolith/Maerl Beds. In *Rhodolith/Maerl Beds: A Global Perspective*; Riosmena-Rodrigues, R., Nelson, W., Aguirre, J., Eds.; Coastal Research Library, Springer: New York, NY, USA, 2017; Volume 15, pp. 55–85.
50. McCoy, S.J.; Kamenos, N.A. Coralline algae (Rhodophyta) in a changing world: Integrating ecological, physiological, and geochemical responses to global change. *J. Phycol.* **2015**. [[CrossRef](#)]
51. Kamenos, N.; Cusack, M.; Moore, P. Coralline algae are global palaeothermometers with biweekly resolution. *Geochim. Cosmochim. Acta.* **2008**, *72*, 771–779. [[CrossRef](#)]
52. Nelson, W. Calcified macroalgae—critical to coastal ecosystems and vulnerable to change: A review. *Mar. Freshw. Res.* **2009**, *60*, 787–801. [[CrossRef](#)]
53. Amado-Filho, G.M.; Moura, R.L.; Bastos, A.C.; Salgado, L.T.; Sumida, P.Y.G.; Guth, A.Z.; Francini-Filho, R.B.; Pereira-Filho, G.H.; Abrantes, D.P.; Brasileiro, P.S.; et al. Rhodolith beds are major CaCO₃ bio-factories in the Tropical South West Atlantic. *PLoS ONE* **2012**, *7*, e35171. [[CrossRef](#)]
54. Amado-Filho, G.M.; Pereira-Filho, G.H.; Bahia, R.G.; Abrantes, D.P.; Veras, P.C.; Matheus, Z. Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil. *Aquat. Bot.* **2012**, *101*, 41–45. [[CrossRef](#)]
55. Amado-Filho, G.M.; Bahia, R.G.; Mariath, R.; Jesionek, M.B.; Moura, R.L.; Bastos, A.C.; Pereira-Filho, G.; Francini-Filho, R.B. Spatial and temporal dynamics of the abundance of crustose calcareous algae on the southernmost coral reefs of the western Atlantic (Abrolhos Bank, Brazil). *Algae* **2018**, *33*, 85–99. [[CrossRef](#)]
56. Holz, V.L.; Bahia, R.G.; Karez, C.S.; Vieira, F.V.; Moraes, F.C.; Vale, N.F.; Sudatti, D.B.; Salgado, L.T.; Moura, R.L.; Amado-Filho, G.M.; et al. Structure of Rhodolith Beds and Surrounding Habitats at the Doce River Shelf (Brazil). *Diversity* **2020**, *12*, 75. [[CrossRef](#)]
57. Morse, D.E.; Morse, A.N.C. Enzymatic characterization of the morphogen recognized by *Agaricia humilis* (scleractinian coral) larvae. *Biol. Bull.* **1991**, *181*, 104–122. [[CrossRef](#)]

58. Harrington, L.; Fabricius, K.; De'ath, G.; Negri, A. Recognition and selection of settlement substrata determine post-settlement survival in corals. *Ecology* **2004**, *85*, 3428–3437. [[CrossRef](#)]
59. Roberts, R.D.; Kaspar, H.F.; Barker, R.J. Settlement of abalone (*Haliotis iris*) larvae in response to five species of coralline algae. *Shellfish. Res.* **2004**, *23*, 975–987.
60. Knoppers, B.; Medeiros, P.R.P.; Souza, W.F.L.; Jennerjahn, T. The São Francisco Estuary, Brazil. In *The Handbook of Environmental Chemistry*; Wangersky, P.J., Ed.; Springer: Berlin/Heidelberg, Germany, 2005; Volume 5, pp. 51–70.
61. Medeiros, P.R.P.; Knoppers, B.; Souza, W.F.L.; Oliveira, E.N. Contribution of suspended matter in the lower São Francisco River (SE/AL), across different hydrologic conditions. *Braz. J. Aquat. Sci. Technol.* **2011**, *15*, 42–53. [[CrossRef](#)]
62. Fontes, L.C.S.; Santos, J.R.; Santos, L.A.; Mendonça, J.B.S.; Santos, M.S. Sedimentos superficiais da plataforma continental de Sergipe-Alagoas. In *Geologia e Geomorfologia da Bacia de Sergipe-Alagoas*; Carneiro, M.E.R., Ed.; Editora UFS; Coleção Projeto Marseal: São Cristóvão, Brazil, 2017; Volume 1, pp. 64–96.
63. Adey, W.H.; Hernández-Kantún, J.; Johnson, G.; Gabrielson, P.W. Dna sequencing, anatomy, and calcification patterns support a monophyletic, subarctic, carbonate reef-forming *Clathromorphum* (Hapalidiaceae, Corallinales, Rhodophyta). *J. Phycol.* **2015**, *51*, 189–203. [[CrossRef](#)]
64. Thiers, B. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. Available online: <http://sweetgum.nybg.org/ih/> (accessed on 16 September 2019).
65. Maneveldt, G.W.; Van der Merwe, E. *Heydrichia cerasina* sp. nov. (Sporolithales, Corallinophycidae, Rhodophyta) from the southernmost tip of Africa. *Phycologia* **2012**, *51*, 11–21. [[CrossRef](#)]
66. Bahia, R.G.; Abrantes, D.P.; Brasileiro, P.S.; Pereira-Filho, G.H.; Amado-Filho, G.M. Rhodolith bed structure along a depth gradient on the northern coast of Bahia State, Brazil. *Braz. J. Oceanogr.* **2010**, *58*, 323–337. [[CrossRef](#)]
67. Chamberlain, Y.M. The genus *Leptophytum* (Rhodophyta, Corallinaceae) in the British Isles with descriptions of *Leptophytum bornetii*, *L. elatum* sp. nov. and *L. laeve*. *Br. Phycol. J.* **1990**, *25*, 179–199. [[CrossRef](#)]
68. Woelkerling, W.J.; Irvine, L.M.; Harvey, A. Growth-forms in non-geniculate coralline red algae (Corallinales, Rhodophyta). *Aust. Syst. Bot.* **1993**, *6*, 277–293. [[CrossRef](#)]
69. Kaewsuralikhit, C.; Maneekat, S.; Noiraska, T.; Patarajinda, S.; Baba, M. First record of *Sporolithon ptychoides* Heydrich (Sporolithales, Corallinophycidae, Rhodophyta) from Thailand. *Cryptogam. Algal.* **2012**, *33*, 265–276. [[CrossRef](#)]
70. Jesionek, M.B.; Bahia, R.G.; Lyra, M.B.; Leão, L.A.B.; Amado-Filho, G.M. Newly discovered coralline algae in Southeast Brazil: *Tectolithon fluminense* gen. et sp. nov. and *Crustaphytum atlanticum* sp. nov. (Hapalidiales, Rhodophyta). *Phycologia* **2020**, *2020*, 1–15. [[CrossRef](#)]
71. Yoon, H.S.; Hackett, J.D.; Bhattacharya, D. A single origin of the peridinin and fucoxanthin containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11724–11729. [[CrossRef](#)]
72. Hughey, J.R.; Silva, P.C.; Hommersand, M.H. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. *J. Phycol.* **2001**, *37*, 1091–1109. [[CrossRef](#)]
73. Freshwater, D.W.; Rueness, J. Phylogenetic relationships of some European Gelidium (Geliadiales, Rhodophyta) species, Based on rbcL nucleotide sequence analysis. *Phycologia* **1994**, *33*, 187–194. [[CrossRef](#)]
74. Tamura, K.; Stecher, G.; Peterson, D.; Filipowski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [[CrossRef](#)]
75. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [[CrossRef](#)] [[PubMed](#)]
76. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [[CrossRef](#)] [[PubMed](#)]
77. Stamatakis, A. RAxML version 8. 2014: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [[CrossRef](#)] [[PubMed](#)]
78. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [[CrossRef](#)] [[PubMed](#)]

79. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogeny trees. *Bioinformatics* **2001**, *17*, 754–755. [[CrossRef](#)]
80. Keats, D.W.; Chamberlain, Y.M. *Sporolithon ptychoides* Heydrich and *S. episporum* (Howe) Dawson: Two coralline red algae (Corallinales, Sporolithaceae). *S. Afr. J. Bot.* **1993**, *59*, 541–550. [[CrossRef](#)]
81. Bahia, R.G. Algas Coralináceas Formadoras de Rodólitos da Plataforma Continental Tropical e Ilhas Oceânicas do Brasil: Levantamento Florístico e Taxonomia. Ph.D. Thesis, Escola Nacional de Botânica Tropical (ENBT), Rio de Janeiro, Brazil, 2014; p. 231.
82. Chelaru, R.; Săsăran, E.; Tâmas, T.; Bălc, R.; Bucur, I.I.; Ples, G. Middle Miocene carbonate facies with rhodoliths from the NW Transylvanian Basin (Vălenii Șomcutei Cave, Romania). *Facies* **2019**, *65*, 1–16. [[CrossRef](#)]
83. Agência Nacional do Petróleo. Available online: <http://webmaps.anp.gov.br/mapas/Lists/DSPAppPages/MapasBrasil.aspx> (accessed on 20 June 2019).
84. EPBR. Available online: <https://epbr.com.br/oferta-permanente-de-areas-de-exploracao-sobe-para-600-blocos/> (accessed on 20 June 2019).
85. Figueiredo, M.A.O.; Coutinho, R.; Villas-Bôas, A.B.; Tâmega, F.T.S.; Mariath, R. Deep-water rhodolith productivity and growth in the southwestern Atlantic. *J. Appl. Phycol.* **2012**, *24*, 487–493. [[CrossRef](#)]



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