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 tetrasporeangia 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 Melvoxonia 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 woolkerlingii 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.

| Sporolithon Species            | psbA | rbcL |
|-------------------------------|------|------|
| S. franciscanum vs Sporolithon sp. (Australia) | 6.5% | 6%   |
| S. franciscanum vs S. eltorensis | 6.1% | 12.3% |
| S. franciscanum vs S. dimotum  | –    | 6.8% |
| S. franciscanum vs Sporolithon 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 para type 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 epithallial 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: *psb*A DNA sequence from the holotype (MT214482) and the sequences from the paratype: *psb*A (KP142752) and *rbc*L (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 (*psb*A and *rbc*L) (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 *psb*A analysis, *S. episporum* and *S. indopacificum* diverge by 2.7% [25]; and in *rbc*L 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 *rbc*L 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 *psb*A 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].

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