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First molecular analysis of the genus *Bryopsis* (Bryopsidales, Chlorophyta) from Brazil, with an emphasis on the Pernambuco coast

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ABSTRACT

The green algal genus *Bryopsis* has simple morphology and high phenotypic plasticity, making it difficult to identify its species based on morphological characteristics alone. This study evaluated the diversity of *Bryopsis* in northeastern Brazil (the State of Pernambuco), based on morphological and molecular data using the markers *tuf*A and *rbc*L. Molecular analyses were incongruent with morphology, demonstrating the existence of cryptic and polymorphic species in the genus. Of the four taxa cited for the area based only on morphological data (*Bryopsis corymbosa*, *B. pennata*, *B. plumosa*, *Bryopsis* sp.), only *B. pennata* was recorded. Typical specimens of *B. pennata* and *B. plumosa* were grouped with low genetic divergence, 0–0.21 % for *tuf*A and no divergence for *rbc*L, indicating that *B. pennata* is an extremely plastic species that includes specimens with morphotype *B. "plumosa"*. *Bryopsis pennata* var. *secunda* is cited for the first time for northeastern Brazil, with divergence from the typical variety of 0.96–1.57 % for *tuf*A and 0.4 % for *rbc*L. This study showed that broader sampling of *Bryopsis* is necessary in order to confirm the taxonomic status of the species referenced for Brazil, whose phenotypic plasticity may cause overestimation of diversity or reveal cryptic species.

Keywords: *Bryopsis*, cpDNA, macroalgae, *rbc*L, taxonomy, *tuf*A

Introduction

The marine macroalgal genus *Bryopsis* (Bryopsidales, Chlorophyta) is one of the most diverse genera of green macroalgae with 138 species names and 84 infraspecific names, of which only 58 species names, eight varieties and five forms are currently accepted taxonomically (Guiry & Guiry 2020). Its representatives constitute a group with a predominantly marine habit, which develops mainly in areas of rocky shores and coral reefs, with temperatures ranging from five to 27 °C (Horta *et al.* 2001), but they also grow in mangroves and estuaries at low salinities (Guiry & Guiry 2020). With a morphology considered simple, species of *Bryopsis* are characterized by non-calcified coenocytic filaments, with erect uniaxial axes with feather-like branches, called fronds, and prostate axes with a variable extension (Wynne 2005; Cremen *et al.* 2019). *Bryopsis* species have ecological importance as primary producers in

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aquatic ecosystems, as well as providing shelter and protection for small invertebrates (Silva 2018). The genus encompasses potentially invasive species that dominate the environment in eutrophic conditions; thus, they are considered excellent bioindicators (Williams & Smith 2007) and can cause green tides (Song *et al.* 2019). Due to the importance of their bioactive compounds, some species, such as *Bryopsis pennata* and *B. plumosa* have pharmacological importance, with antifungal, antibacterial and anticoagulant activities (Ibrahim *et al.* 2017). In addition, its compounds have been used as raw material in studies for treatments of lung and prostate cancer, tumors and acquired immunodeficiency syndrome (AIDS) (Zhang *et al.* 2010; Holdt & Kraan 2011; Lefranc *et al.* 2019) and, in the development of repellents against the mosquito *Aedes aegypti* (Linnaeus 1762) and *A. albopictus* (Skuse 1894), both recognized as vectors of arboviruses such as dengue, chikungunya, zika and yellow fever (Yu *et al.* 2015).

Bryopsis has a global distribution spanning polar to tropical and temperate regions (Guiry & Guiry 2020). For the tropical and subtropical western Atlantic eight species and eight varieties are currently recognized by Wynne (2017). The genus has a wide distribution on the Brazilian coast, extending from Pará state (northern Brazil) to Rio Grande do Sul state (southern Brazil) (INCT 2020), occurring also in the Oceanic Islands of Brazil: Atol das Rocas, Fernando de Noronha, São Pedro and São Paulo, Abrolhos, Trindade and Martim Vaz (Villaça *et al.* 2006). Five infrageneric taxa occur in Brazil, *Bryopsis corymbosa*, *B. hypnoides*, *B. pennata*, *B. pennata* var. *secunda*, and *B. plumosa* (INCT 2020). Five other taxa were reported for the Brazilian coast, *Bryopsis harveyana* for Bahia (Howe 1928), *B. indica* for São Paulo (Gepp & Gepp 1908; Joly 1957), *B. rosea*, with inaccurate data on collection locations (Martius 1833), *Bryopsis rosea* var. *leprieurii* for Rio de Janeiro (Zeller 1876), and *Bryopsis plumosa* var. *leprieurii* for Rio de Janeiro (Luetzelburg 1922-23) and Ceará (Schmidt 1924). However, these five citations were considered as erroneous records by Oliveira-Filho (1977), being assigned to *B. plumosa* (= *Bryopsis plumosa* var. *leprieurii*) or *B. pennata* (the other taxa). Of the five taxa correctly assigned to Brazil, three are cited for the coast of Pernambuco, *B. corymbosa*, *B. pennata* and *B. plumosa* (Accioly 1989; Széchy *et al.* 1989; Cutrim 1990; Pedrini *et al.* 1992; Angeiras 1995; Muñoz *et al.* 1997; Pereira & Accioly 1998; Pereira *et al.* 1996; Sousa & Cocentino 2004; Santos *et al.* 2006; Ribeiro *et al.* 2008; Simões *et al.* 2009; Burgos *et al.* 2009; Soares & Fujii 2012; Barros 2013; Carvalho *et al.* 2013; Silva 2013; Guimaraens *et al.* 2015), besides *Bryopsis* sp. reported by Ribeiro *et al.* (2008), Barros (2013) and Carvalho *et al.* (2013).

Many previous works on *Bryopsis* were focused on vegetative and reproductive morphological characters, and cytogenetic analyses (*e.g.*, Rietema 1975; Wynne 2005). However, due to the large number of species and infraspecific categories, and the difficulty of delimiting them owing to high phenotypic plasticity and overlapping of morphological characters among its representatives, the taxonomy of the genus *Bryopsis* has been problematic and conflicting. Consequently, the species delimitation based on morphological analysis may lead to misidentifications and misapplied names (Krellwitz *et al.* 2001; Wynne 2005; Morabito *et al.* 2010). Likewise, life-cycle and chromosomal analyses contribute to resolution at higher taxonomic levels, making them insufficient to solve taxonomic problems at the specific level (Kapraun & Shipley 1990).

Considering the ecological and economic importance of *Bryopsis*, and the difficulty in properly delimiting its infrageneric and infraspecific categories, besides the recognition of cryptic and polymorphic species, molecular studies using DNA barcode techniques and phylogenetic markers have been fundamental in assessing the diversity of the genus (Krellwitz *et al.* 2001; Lü & Wang 2011; Hollants *et al.* 2013; Tufiño-Velázquez & Pedroche 2019). Plastid markers have been widely used in molecular studies to delimit green algal species, including *Bryopsis*, mainly *tuf*A and *rbc*L genes (Provan *et al.* 2004; Händeler *et al.* 2010; Hall *et al.* 2010; Leliaert *et al.* 2014; Leliaert & Lopez-Bautista 2015; Cremen *et al.* 2019). *Tuf*A marker (elongation factor Tu gene) obtained the best results in the universality of the primers and quality of the sequences, being proposed as a DNA barcode for green algae, and as a phylogenetic marker as well (Saunders & Kucera 2010). Its effectiveness and successfully as DNA barcode for green algae, showing good phylogenetic resolution at generic and specific level having proven by Dijoux *et al.* (2012), Famà *et al.* (2002), Ximenes *et al.* (2017; 2019), followed by the *rbc*L (Rubisco large subunit gene) also considered suitable for phylogenetic reconstruction (Freshwater *et al.* 1994), having been demonstrated a sufficient level of variation to be informative in intergeneric, inter- and intraspecific studies for green and red algae as well (Freshwater & Rueness 1994; Freshwater *et al.* 1994; Oliveira-Carvalho *et al.* 2012; Leliaert *et al.* 2014; Ximenes *et al.* 2017; 2019). Both have been widely used for other genera in the Bryopsidales, such as *Caulerpa*, *Codium*, and *Halimeda* (Lam & Zechman 2006; Oliveira-Carvalho *et al.* 2012; Kazi *et al.* 2013; Belton *et al.* 2014; Ximenes *et al.* 2017; 2019), and also for other green algae (Mccourt *et al.* 2000; Shimada *et al.* 2003).

In this context, considering the morphological plasticity, the presence of cryptic species and the lack of molecular studies for the genus *Bryopsis* in Brazil, this work aimed to contribute to the understanding of the diversity of *Bryopsis* through the application of molecular analyses of the markers *tuf*A and *rbc*L combined with the morphological traits of the species.

Materials and methods

Sampling and morphological analysis

Specimens of *Bryopsis* were collected along the coast of Pernambuco state (PE) (northeastern Brazil, 7º15'45'; 9º28'18" S and 34º48'35"; 41º19'54" W) at 13 sampling sites (Fig. 1), in the intertidal zone during low tide from 2016 to 2017. The area is located in the Western Atlantic Ocean in the Tropical Zone and present warm, transparent and oligotrophic waters (Horta *et al.* 2001). For morphological/molecular studies, each group of fronds was treated as an individual. Samples were stored in absolute ethanol and silica gel for morphological and/or molecular analyses. Part of group of fronds from a same individual was separated for molecular analyses; the remaining material was used for morphological study and pressed as herbarium vouchers.

Morphological characters were analyzed using both stereoscopic microscope Leica S6D (Wetzlar, Germany) and an optical microscope Zeiss Axioskop (Oberkochen, Germany). Photographic documentation of whole specimens and details of the different portions of the thallus and branchlets were made using a stereomicroscope Zeiss Discovery-V8 A1 (Göttingen, Germany) coupled to a digital camera Axiocam 105 Color (Göttingen, Germany), with images analyzed by the software Zeiss ZEN® (Jena, Germany); and an optical microscope Zeiss Axio Scope.A1 (Göttingen, Germany) coupled to a digital camera AxioCam MRc (Göttingen, Germany) using the software Zeiss ZEN®.

Minimum and maximum measurements were taken for morphometric characters from a set of 10 measurements for each analyzed feature in all specimens from different sampling sites. The specimens were deposited at the herbarium Prof. Vasconcelos Sobrinho of the Universidade Federal Rural de Pernambuco (PEUFR). Additional specimens from the PEUFR were also examined.

Molecular analysis

Total DNA was extracted using CTAB (cetyl trimethyl ammonium bromide) protocol as described by Oliveira-Carvalho *et al.* (2012). For polymerase chain reaction (PCR), the *tuf*A marker was amplified using the primer pair *tuf*AF (5' TTGTTC KAACATAAA ATT GWGGTC 3') and *tuf*A_alg_up (5' ATGATWACNGGHGCNGCWCAAATG 3') (Händeler *et al.* 2010), following the cycle described by Händeler *et al.* (2010). For the marker *rbc*L was used the primer pair: F623-603 (5' TCWCAACCHTYTATGCGTTGG 3') (Curtis *et al.* 2008) and R1396-1372 (5' AATTTCTTTCCAAACTTCACAAGC 3') (Lam & Zechman 2006), following the cycle described by Curtis *et al.* (2008). PCR was performed using illustra PuReTaq Ready-To-Go PCR Beads kit (GE Healthcare, Buckinghamshire, UK) following the manufacturer's instructions. The fragments were amplified in a Techne TC-4000 thermocycler (Bibby Scientific Ltd, Staffordshire, UK). All PCR products were analyzed by electrophoresis in 1 % agarose to check product size and were purified using the GFXTM PCR DNA and Gel Band Purification kit (GE Healthcare, Buckinganshire, UK), following the manufacturer's instructions. Purified amplicons for both markers were sequenced

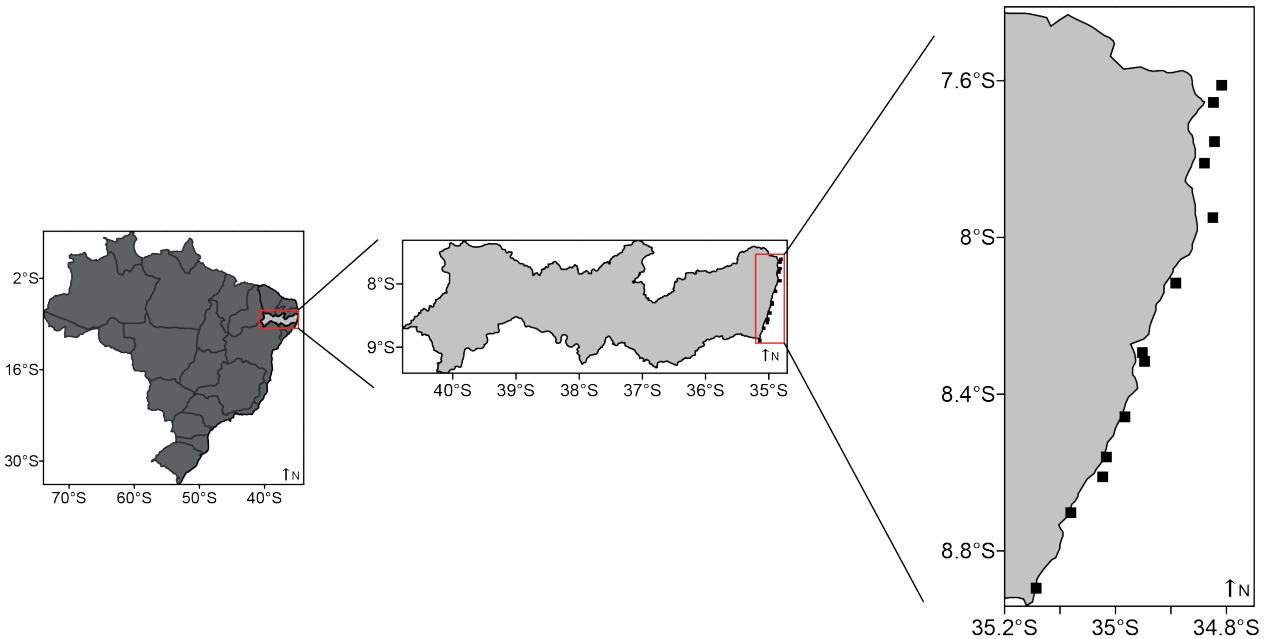

Figure 1. Map showing the sampling sites of *Bryopsis* along the Pernambuco coast (northeastern Brazil).

using the BigDyeTM Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, USA), with the same primers of PCR on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Sequences were checked using the BlastN algorithm, through the NCBI online platform (Altschul *et al.* 1997). Consensus sequences and multiple alignments for both *tuf*A and *rbc*L sequences were constructed using the computer program BioEdit v7.0.4.1 software (Hall 1999). For each marker, a matrix was created with the sequences generated in this study plus those available in the GenBank database used in the analyses (Tab. S1 in supplementary material).

Molecular analyzes were performed using the Neighbor-Joining (NJ) distance method in PAUP 4.0b10 program (Swofford 2002) with 2000 boostrap replicates. For phylogenetic analysis, the most appropriated model of sequence evolution for maximum likelihood (ML) and Bayesian inference (BI) was selected using jModeltest v2.1.10 (Darriba *et al.* 2012) under the Akaike Information Criterion (AIC) implemented on the online server CIPRES Science Gateway v3.3 (Miller *et al.* 2011). The model selected for *tuf*A and *rbc*L was the general time-reversible model of nucleotide substitution with invariant sites and gamma distributed rates for the variable sites (GTR + I + G). The maximum likelihood analysis (ML) was performed using IQ-Tree v1.4.3 (Nguyen *et al.* 2015) with 1000 boostrap replicates on the IQ-Tree web portal. Bayesian Inference (BI) was performed using MrBayes v.3.2.2 program (Ronquist *et al.* 2012). For BI analysis, two runs with four chains of the Markov chain Monte Carlo (one hot and three cold) were used, sampling one tree every 1,000 generations for 4,000,000 generations, starting with a random tree. We discarded the first 50,000 generations in both runs as the burn-in to build the consensus tree. After discarding the trees associated with "burn-in", a consensus tree was built for both *tuf*A and *rbc*L markers. In all analyses, gaps were considered as missing data. For both *tuf*A and *rbc*L matrices, the percentages of intraspecific and interspecific divergences were calculated using uncorrected '*p*' distances in PAUP.

Results

Molecular analysis

Thirty-one new *Bryopsis* sequences were obtained from specimens collected on the Pernambuco coast, 19 sequences for *tuf*A and 12 sequences for *rbc*L. For the *tuf*A marker, 53 sequences were used with an alignment of 733 bp, and of the 34 sequences obtained from GenBank, three were used as outgroups, *Codium tomentosum* Stackhouse (KX855797), *Pseudoderbesia arbuscula* E.Calderon & R.Schnetter (MH591099) and *Derbesia* sp. (KU361860) (Tab. S2 in supplementary material).

The *tuf*A analyses recover *Bryopsis* as monophyletic, with high support for NJ and moderate for ML (Fig. 2). The Brazilian sequences formed two subclades with high ML bootstrap support and high Bayesian posterior probability (PP). The major subclade grouped most of the Brazilian samples, identified as *B.* "*plumosa*" and *B. pennata*, with high support of ML and PP, and in which three other unidentified *Bryopsis* sequences (*Bryopsis* sp. from Oahu, USA, and *Bryopsis* sp.1, collected from a marine aquarium) were grouped. Except for the sample *B.* "*plumosa*" (102), all sequences of this subclade were 100 % identical, including *Bryopsis* sp. and *Bryopsis* sp.1 from GenBank. The divergence between the sample 102 and others of this subclade ranged from 0.15 % (102 *vs.* *B.* sp. and *B.* sp. 1) to 0.21 % (102 *vs.* 129). The low divergence of this subclade (0-0.21 %) indicates that all samples belong to the same genetic species. The second subclade was formed by two Brazilian samples of *B. pennata* var. *secunda* grouped with an unidentified sample of *Bryopsis*, named as *B.* sp. 28 from the Philippines, all 100 % identical, and with high to moderate support. The lack of genetic divergence between the sequences of this subclade supports the conclusion that they all correspond to the same genetic species. These two subclades, *B.* "*plumosa*"-*B. pennata* - *B.* sp.1 - *B.* sp. and *B. pennata* var. *secunda* - *B.* sp.28, differed from each other from 0.96 % to 1.57 %. For unidentified samples downloaded from GenBank that were grouped in these two subclades (*B* sp., *B.* sp.1 and *B.* sp.28), there are no descriptions (Leliaert *et al.* 2014; Wade & Sherwood 2017) that allow morphological comparison with the Brazilian material.

Four *tuf*A sequences of *Bryopsis plumosa* from GenBank were included in the analyses, and this species was shown to be paraphyletic (Fig. 2). The sample of *B. plumosa* from Argentina (JQ755423) was not grouped with the others from France (LN810504, FJ432653) and with another whose sampling site was not specified (U09424). These latter three sequences were split into two subclades (Fig. 2). The sequence of France (LN810504, Traon Erch, Brittany) corresponds to the complete sequencing of the chloroplast genome done by Leliaert & Lopez-Bautista (2015), and its sampling site corresponds to a region near to the type locality (Exmouth, Devon, England). The sequence LN810504 formed a subclade with *Bryopsis* sp. from the USA (HQ610244), being 100 % identical, and with a more divergent sequence of *B. corymbosa* from France (1.6 %). The other *Bryopsis plumosa* sequence from France (FJ432653, Pas de Calais, Boulogne-sur-Mer), generated by Verbruggen *et al.* (2009), is also near the type locality; however, it formed another subclade with *B. plumosa* (U09424), whose divergence between them was 0.99 %. The two sequences of *B. plumosa* from France showed high divergence (3.3 %), indicating that that belong to separate genetic species. The sample of *B. plumosa* from Argentina (JQ755423) proved to be highly divergent from the other sequences of *B. plumosa*, varying from 7.0 % to 7.2 % (Tab. 1).

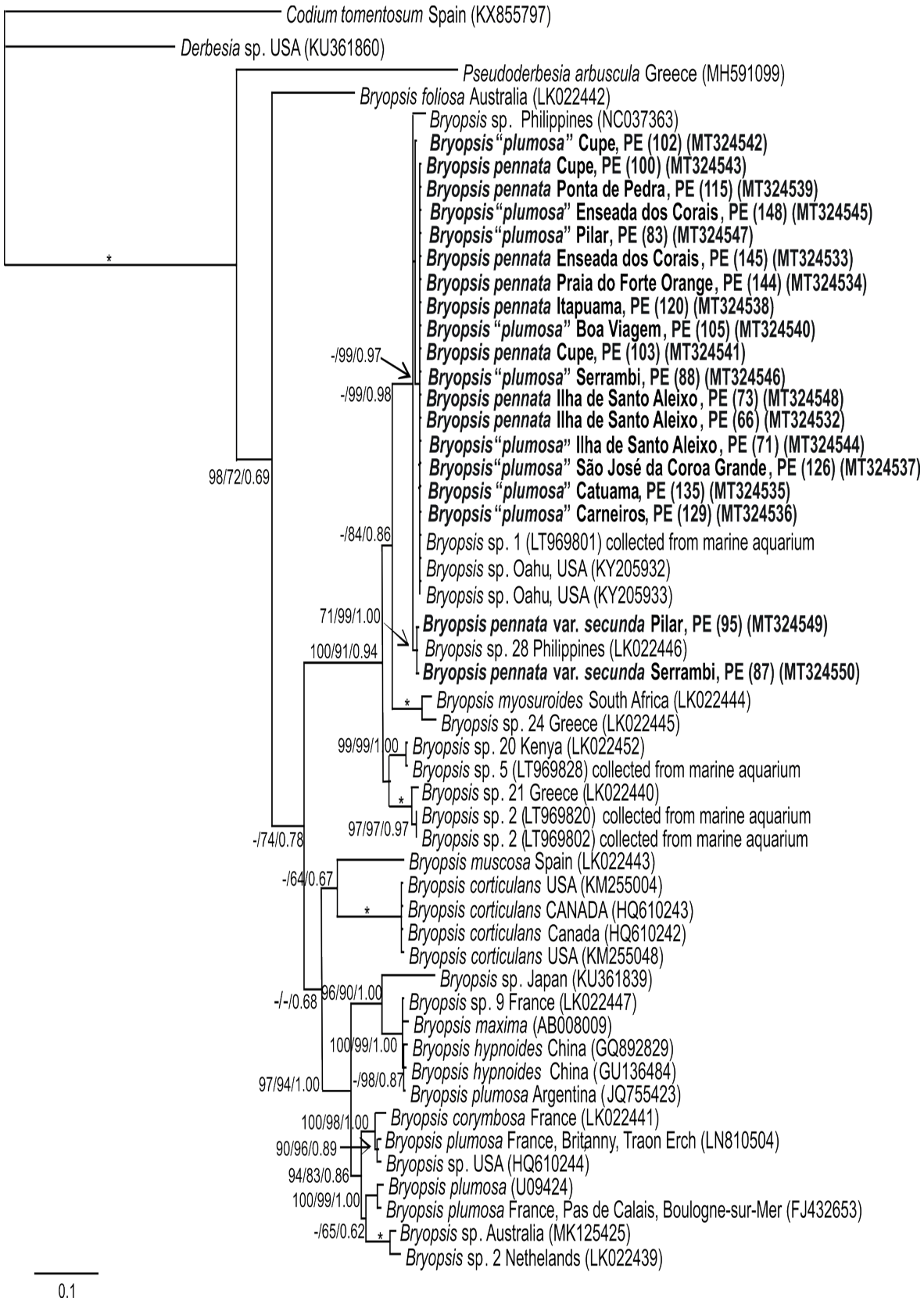

Figure 2. Consensus tree derived from Bayesian Inference of *tuf*A sequences for *Bryopsis* taxa. NJ/ML/PP values are indicated on the branches. Only values above 60 were considered. Samples generated in this study are in bold; the number following these sequences referred to field id; - indicates lack of bootstrap support or values under 60; *indicates full support. The scale bar represents the genetic distances in substitutions per nucleotide.

Table 1. Genetic divergence values (%) of *Bryopsis pennata* from the Pernambuco coast and samples of *B. plumosa* from GenBank for the *tuf*A marker. * Samples of *B. pennata*, *1 *B. plumosa*.

Táxon	100	115	148	83	145	144	120	105	103	88	73	66	71	126	135	129	102	LT969801	KY205932	KY205933	JQ755423	LN810504	FJ432653	U09424
100*	ID																							
115*	0	ID																						
148*	0	0	ID																					
83*	0	0	0	ID																				
145*	0	0	0	0	ID																			
144*	0	0	0	0	0	ID																		
120*	0	0	0	0	0	0	ID																	
105*	0	0	0	0	0	0	0	ID																
103*	0	0	0	0	0	0	0	0	ID															
88*	0	0	0	0	0	0	0	0	0	ID														
73*	0	0	0	0	0	0	0	0	0	0	ID													
66*	0	0	0	0	0	0	0	0	0	0	0	ID												
71*	0	0	0	0	0	0	0	0	0	0	0	0	ID											
126*	0	0	0	0	0	0	0	0	0	0	0	0	0	ID										
135*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ID									
129*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ID								
102*	0.16	0.17	0.16	0.16	0.16	0.20	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.20	0.18	0.21	ID							
LT969801*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.15	ID						
KY205932*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.15	0	ID					
KY205933*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.15	0	0	ID				
JQ755423 (Argentina)*1	14.8	15.5	14.9	14.7	15.2	16	15.1	15	15.1	14.9	14.8	14.8	15.5	16	15.5	16.2	14.1	14.2	14.3	14.3	ID			
LN810504 (France)*1	12.4	12.7	12.4	12.4	12.6	13.2	12.5	12.5	12.5	12.5	12.4	12.4	12.6	13.2	13	13.6	12.2	12.3	12.4	12.4	7.1	ID		
FJ432653 (France)*1	12.4	12.8	12.5	12.4	12.7	13.4	12.7	12.6	12.7	12.5	12.4	12.4	12.8	13.4	13.2	13.8	12	12.2	12.2	12.2	7	3.3	ID	
U09424*1	12.8	13.3	13	12.8	13.2	13.8	13.1	13.1	13.1	13	12.9	12.9	13.3	13.8	13.6	14.1	12.5	12.6	12.7	12.7	7.2	3.5	0.99	ID

The samples identified as *B. "plumosa"* collected at different sites along the Pernambuco coast grouping with *Bryopsis pennata* samples (see above) were highly divergent from both *B. plumosa* from France, varying from 12.2 % to 13.6 % from LN810504, and from 12 % to 13.8 % from FJ432653, although morphologically typical plants of this species have been observed.

The eight samples identified as *Bryopsis pennata* positioned in this same clade, also with wide distribution on the Pernambuco coast, could not be compared molecularly with any other *B. pennata tuf*A sequence because there are no sequences of this marker available in the databases. With the possibility of citing *B. plumosa* among our samples being discarded by molecular data, we maintained the identification of these samples as *B. pennata*, also supported by morphological analysis, with typical plants observed in different samples. Our results showed that *B. pennata* is extremely polymorphic, whereas the genetic divergence between samples was very low. Samples of *B. pennata* var. *secunda* also diverged in high percentage values from the *B. plumosa* from France (12.5-12.8 %, Tab. 2). The interspecific divergence observed between Brazilian samples and those from GenBank considered in this study ranged from 3.3-16.2 % for *tuf*A.

For *rbc*L, 66 sequences were used with an alignment of 1284 bp. Of the 54 sequences obtained from GenBank, *Pseudoderbesia* sp. (LK022434) was used as an outgroup (Fig. 3). The genetic divergences presented for *rbc*L were calculated on the final alignment of 1284 bp, from which the consensus tree was generated (Fig. 3). A second *rbc*L alignment was constructed with a larger number of *Bryopsis* sequences available on GenBank in order to ascertain the phylogenetic position of Brazilian samples using a larger sampling. These alignments consisted of a total of 147 sequences and, due to many sequences being partial for this marker, a shorter alignment with 689 bp was generated, from which an ML analysis was made (Fig. S1 in supplementary material). The results obtained in the two analyses were similar, with the Brazilian samples showing the same phylogenetic position (Fig. 3, Fig. S1 in supplementary material).

The *rbc*L consensus tree (Fig. 3) showed that the monophyly of *Bryopsis* was not supported by this gene. However, Brazilian sequences were grouped similarly to the results obtained with *tuf*A, with high support for all analyses. The samples identified as *B. pennata* and *B. "plumosa"* are 100 % identical. The two Brazilian sequences of *B. pennata* var. *secunda* (100 % identical) were grouped into a subclade with two GenBank sequences, namely *Bryopsis* sp. 28, one from Tanzania (HF583394) and another from Kenya (HF583393), with high support for ML and PP. Brazilian samples of *Bryopsis pennata-B. "plumosa"* diverged from the var. *secunda* by 0.4 %. The divergence within the subclade formed by *B. pennata* var. *secunda* and *Bryopsis* sp. 28 was very low, ranging from 0 % (between *B. pennata* var. *secunda* and *B.* sp. 28 from Tanzania) to 0.15 % (between *B. pennata* var. *secunda* and *B.* sp. 28 from Kenya), indicating that they are the same genetic species. The genetic divergence between samples of *B. pennata-B. "plumosa"* and *B. pennata* var. *secunda-B.* sp. 28) for *rbc*L ranged from 0.27-0.4 %, being lower than that observed with *tuf*A (Tab. 3).

Eight *rbc*L sequences of *Bryopsis plumosa* from GenBank were included in the analyses (Fig. 3). These sequences formed four distinct clades, indicating that *B. plumosa* is not monophyletic, corroborating the results of *tuf*A. Among the eight sequences analyzed, one is from Traon Erch, Brittany, France (LN810504), a region near to the type locality (Exmouth, Devon, England). The genetic divergence between replace by this sample from France and the Brazilian ones (*B. "plumosa"-B. pennata*) was high, 8.0 %, whereas the divergence with the samples of *B. pennata* var. *secunda* was slightly higher, 8.6 %. Another sequence of *B. plumosa* from France near the type locality (Pas de Calais, Boulogne-sur-Mer, FJ432637, Verbruggen *et al.* 2009) was positioned in a distinct clade, and diverged from *B. "plumosa"-B. pennata* by 6.7-6.9 % and from *B. pennata* var. *secunda* by 7.1-7.2 %. The genetic divergences observed between the Brazilian material and the *B. plumosa* samples from GenBank from different regions (France, Australia, Argentina and Japan) are shown in Table 3.

Table 2. Genetic divergence values (%) of *Bryopsis pennata* var. *secunda* from the Pernambuco coast and samples of *B. plumosa* from GenBank for the *tuf*A marker. *Samples of *B. pennata* var. *secunda*, *1 *B. plumosa*, and *2 related samples.

Taxa	95	LK022446	87	JQ755423	LN810504	FJ432653	U09424	HQ610244	MK125425	LK022439	LK022441
95*	ID										
LK022446*	0	ID									
87*	0	0	ID								
JQ755423 (Argentina)*1	15	14	14.8	ID							
LN810504 (France)*1	12.6	12.3	12.6	7.1	ID						
FJ432653 (France)*1	12.8	12.5	12.5	7	3.3	ID					
U09424*1	13.3	12.6	13	7.2	3.5	0.99	ID				
HQ610244 *B.* sp. (USA)*2	12.7	12.6	12.6	7.4	0	3.3	3.7	ID			
MK125425 *B.* sp. (Australia)*2	13.9	13.1	13.8	8	4.2	4.3	4.8	4.5	ID		
LK022439 *B.* sp.2 (Nethelands)*2	14	13.6	13.9	8.6	4.8	4.4	5.2	5	1.5	ID	
LK022441 *B. corymbosa* (France)*2	13.4	13	13.3	7.7	1.4	4	4.1	1.6	5	5.2	ID

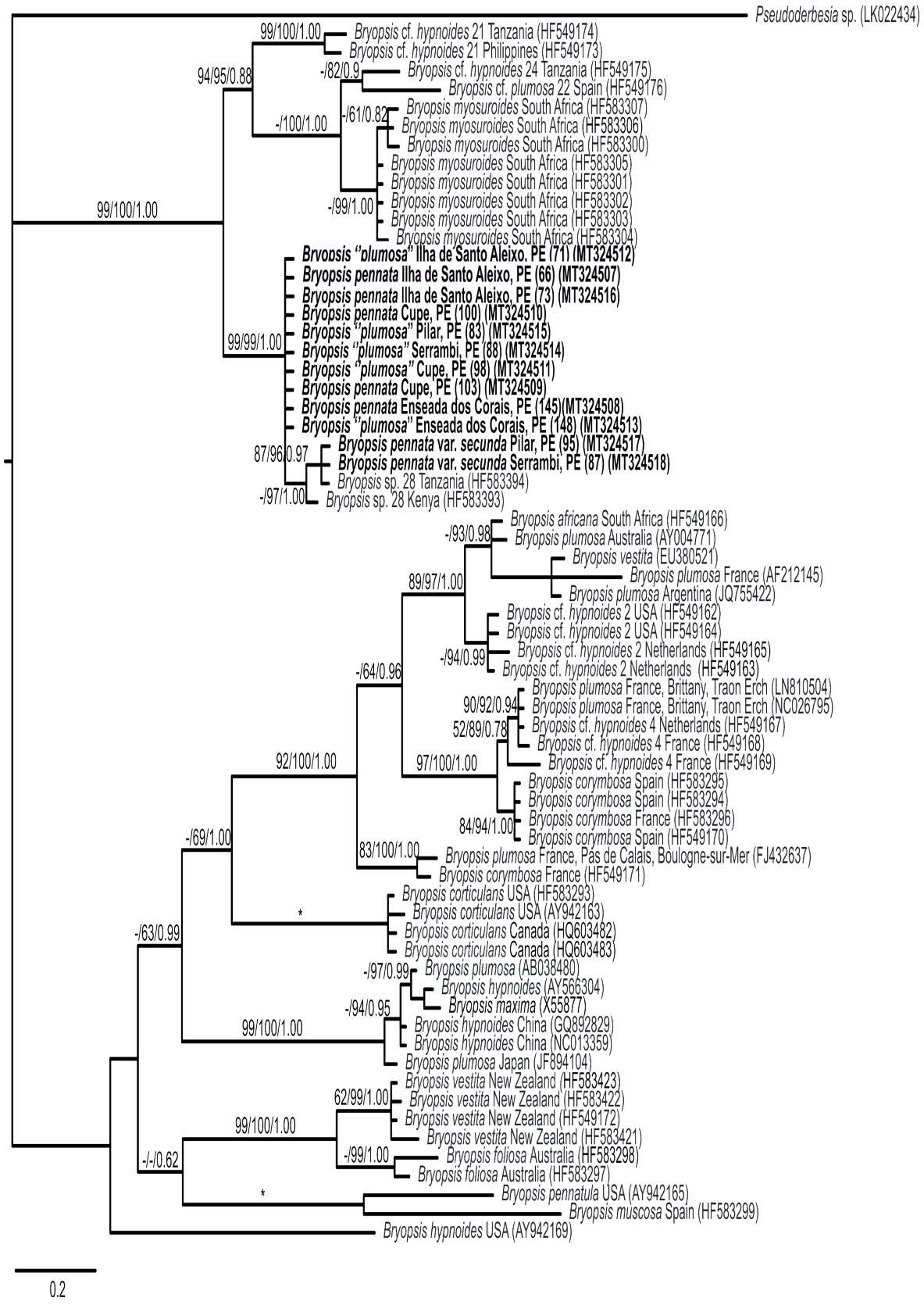

Figure 3. Consensus tree derived from Bayesian Inference of *rbc*L sequences for *Bryopsis* taxa. NJ/ML/PP values are indicated on the branches. Only values above 60 were considered. Samples generated in this study are in bold; the number following these sequences referred to field id; - indicates lack of bootstrap support or values under 60; *indicates full support. The scale bar represents the genetic distances in substitutions per nucleotide.

Table 3. Genetic divergence values (%) of *Bryopsis pennata* from the Pernambuco coast and samples of *B. plumosa* from GenBank for the *rbc*L marker. *Samples of *B. pennata*; *[1]*B. pennata* var. *secunda*; and *[2]*Bryopsis plumosa*.

Taxa	71	66	73	100	83	88	98	103	145	148	95	87	HF583394	HF583393	AY004771	AF212145	JQ755422	LN810504	NC026795	FJ432637	AB038480	JF894104
71*	ID																					
66*	0	ID																				
73*	0	0	ID																			
100*	0	0	0	ID																		
83*	0	0	0	0	ID																	
88*	0	0	0	0	0	ID																
98*	0	0	0	0	0	0	ID															
103*	0	0	0	0	0	0	0	ID														
145*	0	0	0	0	0	0	0	0	ID													
148*	0	0	0	0	0	0	0	0	0	ID												
95*1	0.46	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	ID											
87*1	0.47	0.44	0.4	0.44	0.44	0.44	0.45	0.45	0.45	0.45	0	ID										
HF583394 (Tanzania)*1	0.42	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0	0	ID									
HF583393 (Kenya)*1	0.28	0.27	0.3	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.15	0.15	0.24	ID								
AY004771 (Australia)*2	7.6	7.4	7.5	7.5	7.5	7.4	7.4	7.6	7.6	7.6	8	7.9	7.4	7.3	ID							
AF212145 (France)*2	10.1	11.1	11	11.1	11.1	11	11.2	11.3	11.3	11.3	11	11.6	9.4	9.2	2.3	ID						
JQ755422 (Argentina)*2	8.1	7.8	7.8	7.8	7.8	7.6	7.8	8	8	8	8.3	8.3	7.2	7.1	0	0	ID					
LN810504 (France)*2	8.4	8.2	8.1	8.1	8.1	8	8	8.3	8.3	8.3	8.6	8.6	7.6	7.4	2.3	4.4	2.6	ID				
NC026795 (France)*2	8.4	8.2	8.1	8.1	8.1	8	8	8.3	8.3	8.3	8.6	8.6	7.6	7.4	2.3	4.4	2.6	0	ID			
FJ432637 (France)*2	6.9	6.8	6.7	6.7	6.7	6.7	6.8	6.8	6.8	6.8	7.2	7.1	7.3	7.3	2.1	7.2	2.3	2.1	2.1	ID		
AB038480*2	7.4	7.1	7	7	7	6.9	7.1	7.2	7.2	7.2	7.5	7.4	7.1	6.9	5.9	7.9	6.2	5.5	5.5	5	ID	
JF894104 (Japan)*2	7.5	7.3	7.3	7.3	7.3	7.2	7.3	7.5	7.5	7.5	7.7	7.7	6.9	6.7	5.6	6.7	6.1	5.3	5.3	5.1	0.32	ID

An *rbc*L sequence of *Bryopsis pennata*, with 562 bp (DQ469323) is available in GenBank. This sample was collected in the Virgin Islands (St. Thomas) corresponding, therefore, to a sequence of the type locality (Caribbean Sea). However, the partially amplified region of this sequence was not coincident with our sequences, not allowing a comparison between them. The intraspecific divergence observed for different taxa included in the analysis with *rbc*L was low. There was no genetic divergence between the four sequences *of B. corymbosa* from Spain and France (Fig. 3); clades formed by eight *B. myosurioides* sequences from South Africa, and four *B. vestita* sequences from New Zealand showed both 0-0.2 % of divergence; the clade with four *B.* cf. *hypnoides* 2 sequences from USA and Netherlands ranged from 0.08-0.2 %, while the four sequences that formed the *B. corticulans* clade from USA and Canada showed slightly higher intraspecific divergence, 0-0.3 %. However, the range of intraspecific variation for these taxa, collected in different geographical areas (0-0.3 %), remained below, although close to the limit of the divergence observed between the Brazilian sequences of *B. pennata* and *B. pennata* var. *secunda*, whose genetic divergence was 0.4%. Despite the lower divergence observed between these two taxa for *rbc*L (0.4 %) compared to *tuf*A (0.96-1.57 %), the clades formed by *B. pennata* and *B. pennata* var. *secunda* were the same for both markers. The interspecific divergence observed between Brazilian samples and those from GenBank considered in this study ranged from 6.7-11.6 % for *rbc*L (Tab. 3).

Morphological analysis

Based on molecular analyses, two *Bryopsis* taxa were identified for the Pernambuco coast: *B. pennata* and *B. pennata* var. *secunda*. Twenty-one samples were examined; 19 were identified as *B. pennata* (including the morphotype *B.* "*plumosa*") and two as *B. pennata* var. *secunda*. A comparison of the morphological characters for these taxa is shown in Table 4.

Bryopsis pennata J.V.Lamouroux var. ***pennata*** Nouv. Bull. Sci. Soc. Phil. Paris. 1: 333 (Lamouroux 1809).

(Figs. 4A-O, 5A-O).

Homotypic synonym: *Bryopsis plumosa* var. *pennata* (J.V.Lamouroux) Børgesen

Heterotypic synonym: *Bryopsis densa* Pilger.

Type locality: Antilles, West Indies (Caribbean Sea).

For list of material examined and additional material examined see List S1 in supplementary material.

Description: Erect plants, forming small dense tufts, up to 8.0 cm high (Fig. 4A). Filamentous, coenocytic, cylindrical, flaccid in texture, dark green to light green in color, attached to the substrate by rhizoids. Frond with a linear to lanceolate outline (Fig. 4C-D). Erect filaments usually with an order of lateral branches (branchlets) of approximately uniform length along the axis, gradually smaller towards the apex of the thallus (Fig. 4E-I). Filaments generally naked in the lower portion of the thallus and with opposite branchlets, distichous or slightly alternate in the upper third, in some cases with discontinuous branching (Fig. 4J), or with branchlets arranged unilaterally (Fig. 4M). Cylindrical branchlets, without septa, constricted at the base and with obtuse apex, measuring 402-510 µm in length in the basal portions, 400-480 µm in the middle, and 20-44 µm in the apical portions. Fertile specimens not observed.

Habitat: Species very common on the Pernambuco coast. Specimens were collected in the intertidal zone, the two morphotypes (*B. pennata* and *B. "plumosa"*) often found in mixed populations, growing on consolidated substrate (sandstone reefs). Some specimens were collected growing on *Halimeda opuntia* (Linnaeus) J.V.Lamouroux, *Bryothamnion triquetrum* (S.G.Gmelin) M.Howe, *Gelidiella acerosa* (Forsskål) Feldmann & Hamel, *Palisada perforata* (Bory) K.W.Nam, *Padina gymnospora* (Kützing) Sonder, *Dictyota* sp. and *Sargussum polyceratium* Montagne.

References for Pernambuco: Accioly (1989), Széchy *et al.* (1989), Cutrim (1990), Pedrini *et al.* (1992), Angeiras (1995), Pereira *et al.* (1996), Muñoz *et al.* (1997), Pereira & Accioly (1998), Sousa & Cocentino (2004), Santos *et al.* (2006), Ribeiro *et al.* (2008), Burgos *et al.* (2009), Soares & Fujii (2012), Barros (2013), Carvalho *et al.* (2013), Silva (2013), Guimaraens *et al.* (2015). As ***B. plumosa*:** Accioly (1989), Széchy *et al.* (1989), Cutrim (1990), Angeiras (1995), Simões *et al.* (2009), Burgos *et al.* (2009), Barros (2013), Carvalho *et al.* (2013), Silva (2013), Guimaraens *et al.* (2015), Soares & Fujii (2012).

Remarks: Plants of *Bryopsis pennata* showed great phenotypic plasticity. Samples previously identified as *B. plumosa* (Fig. 5A-O) based on morphological data were grouped in the same clade as *B. pennata* by the two markers used (*tuf*A and *rbc*L), with low intraspecific divergence. Plants with morphotype *B.* "*plumosa*" (Fig. 5A-O) are densely tufted, up to 12 cm high (Fig. 5A), with more dense branching in the apical portions (Fig. 5C-D), or even scarce of very unequal size. Characteristically, the fronds have a pyramidal outline, caused by the decrease in the length of the branchlets towards the apex (Fig. 5F-G). Erect filaments with one or more orders of branches distichously pinnate to bipinnate, or with slightly alternating, irregular or discontinuous branching. Branchlets are often longer in morphotype *B.* "*plumosa*", measuring 462-1040 µm in length in the basal portions, 574-1240 µm in the middle, and 354-480 µm in the apical portions (Tab. 4).

Bryopsis pennata* var. *secunda (Harvey) Collins & Hervey, Proc. Am. Acad. Arts Sci: 62. 1917.

(Fig. 6A-J)

Basionym: *Bryopsis plumosa* var. *secunda* Harvey (1858)

Heterotypic synonym: *Bryopsis harveyana* J.Agardh

Type locality: Key West, Florida, USA.

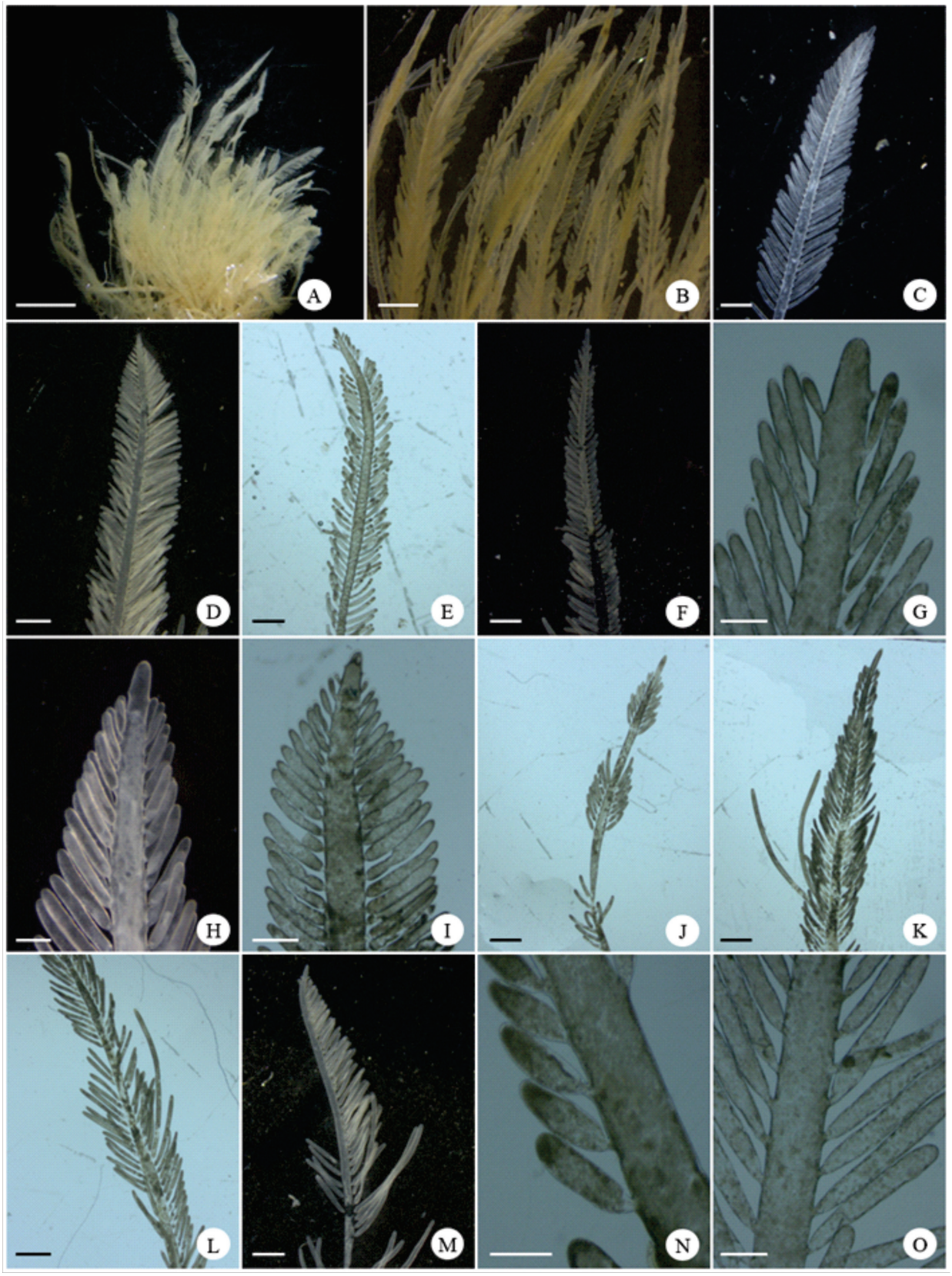

Figure 4. *Bryopsis pennata*. **A.** Habit of the thallus. **B-F.** Detail of fronds with regular distribution of branchlets. **G-I.** Detail of the variation of the apices of the thallus. **J.** Detail of a frond with discontinuous branchlets. **K-L.** Detail of fronds with irregular branchlets. **M.** Detail of a frond with unilateral branchlets. **N-O.** Detail of the distribution of the branchlets. Note branchlets constricted at the base. Bars: **A** = 1 cm, **B-F**, **J-M** = 1,000 µm, **G-I**, **N-O** = 200 µm.

Description: Erect plants forming dense tufts up to 2.0 cm high (Fig. 6A-B). Filamentous, coenocytic, cylindrical, flaccid in texture, dark green in color, attached to the substrate by rhizoids. Frond formed by two rows of branchlets with unilateral arrangement, often with the apices incurved (Fig. 6H). Erect filaments with an order of lateral branches (branchlets) of approximately uniform length, gradually smaller towards the apex (Fig. 6C-F). Filaments commonly naked in the lower third of the thallus and with opposite branchlets, distichous e/or alternate in the upper third of the thallus (Fig. 6E-F). Cylindrical branchlets, without septa, constricted at the base and with obtuse apex, measuring 415-480 µm in length in the basal portions, 560-610 µm in the middle, and 312-340 µm in the apical portions. Fertile specimens not observed.

Material examined: BRAZIL. Pernambuco: Pilar, 07°45'20.04" S - 34°49'17.25" W, IV 2017, *M.F.O. Carvalho* (PEUFR55185); Serrambi, 08°33'37.35" S - 35°0'58.91" W, IV 2017, *M.F.O. Carvalho* (PEUFR55192).

Remarks: *Bryopsis pennata* var. *secunda* is easily confused with young thalli of *B. pennata* due to its small size; however, it presents distinctive features such as apices of the fronds incurved and two rows of branchlets overlapping unilaterally. There is no record of this taxon for the north and northeast of Brazil, only for the states of Espírito Santo, Paraná and Santa Catarina (INCT 2020). Our examples agree morphologically with the records of Silva *et al.* (1996); Russell (2000); Coppejans *et al.* (2004). This is its first citation for northeastern Brazil.

Discussion

Previous studies on the genus *Bryopsis* for the Pernambuco coast, as well as for the entire Brazilian coast, were based exclusively on morphological data (Pereira & Accioly 1998). The present work constitutes the first molecular study of *Bryopsis* for Brazil, in which the first sequences of *tuf*A and *rbc*L were generated from material collected on the Pernambuco coast. Two species were previously cited for the state of Pernambuco, *B. pennata* and *B. plumosa* (*e.g.*, Pereira & Accioly 1998; Carvalho *et al.* 2013; Barros 2013). Different authors have reported the difficulty in delimiting *Bryopsis* species due to the high phenotypic plasticity and the overlapping of morphological characters, leading them to quote their specimens without specific identification, as *Bryopsis* sp. (Ribeiro *et al.* 2008; Burgos 2009; Barros 2013; Carvalho *et al.* 2013). The occurrence of *B. corymbosa* for Pernambuco coast (Gaibu Beach, det. Y. Ugadim, SPF-Algae 9527) may be a misidentification, in that there is only a single record based on morphological data. In addition, this species may be confused with the morphotype *B. "plumosa"* because they share morphological characteristics such as the irregular size of the branchlets and the branches with irregular growth. However, they differ by the gradual decrease of the branchlets length towards the apex, giving a pyramidal outline to the thallus in *B. plumosa*, and by the gradual decrease of the branchlets length towards the apex, giving a corymbose outline in

Table 4. Comparison of morphological characters of *Bryopsis* J.V.Lamouroux from the Pernambuco coast (northeastern Brazil). D = dense, ND = not dense, PC = present continuous; PD = present discontinuous; A= absence.

Field code	Taxa	Thallus			Branchlets				
					Presence		Length (µm)		
		Height (cm)	Density of tufts	Orders of branches	Upper third of the thallus	Lower third of the thallus	Basal	Middle	Apical
66	*Bryopsis pennata*	7	D	>1	PC	PD	505	478	32
71	*Bryopsis "plumosa"*	12	ND	> 1	PD	A	1040	1198	480
73	*Bryopsis pennata*	8	D	1	PC	PD	510	462	44
76	*Bryopsis "plumosa"*	4	ND	>1	PC	PD	860	980	360
83	*Bryopsis "plumosa"*	9	ND	>1	PD	A	872	1010	457
87	*Bryopsis pennata* var. *secunda*	2	ND	1	PC	A	415	610	312
88	*Bryopsis "plumosa"*	9	ND	>1	PD	PD	954	1240	422
95	*Bryopsis pennata* var. *secunda*	1.5	ND	1	PC	A	480	560	430
98	*Bryopsis "plumosa"*	8	ND	>1	PC	PD	790	1017	452
100	*Bryopsis pennata*	2	D	1	PC	PC	402	400	20
102	*Bryopsis "plumosa"*	3	D	1	PC	A	462	574	417
103	*Bryopsis pennata*	7	D	>1	PC	PD	502	478	37
105	*Bryopsis "plumosa"*	8	D	>1	PD	PD	815	1124	473
115	*Bryopsis pennata*	6	D	>1	PD	PD	506	480	41
120	*Bryopsis pennata*	4.5	D	1	PC	PD	430	431	25
126	*Bryopsis "plumosa"*	7	D	>1	PC	A	773	998	440
129	*Bryopsis "plumosa"*	9.5	ND	>1	PD	PD	1002	1195	454
135	*Bryopsis "plumosa"*	8	D	>1	PD	PD	905	1134	383
144	*Bryopsis pennata*	4.5	D	1	PC	PD	427	437	25
145	*Bryopsis pennata*	2.5	ND	1	PC	PC	412	423	23
148	*Bryopsis "plumosa"*	5	ND	>1	PC	PD	768	963	354

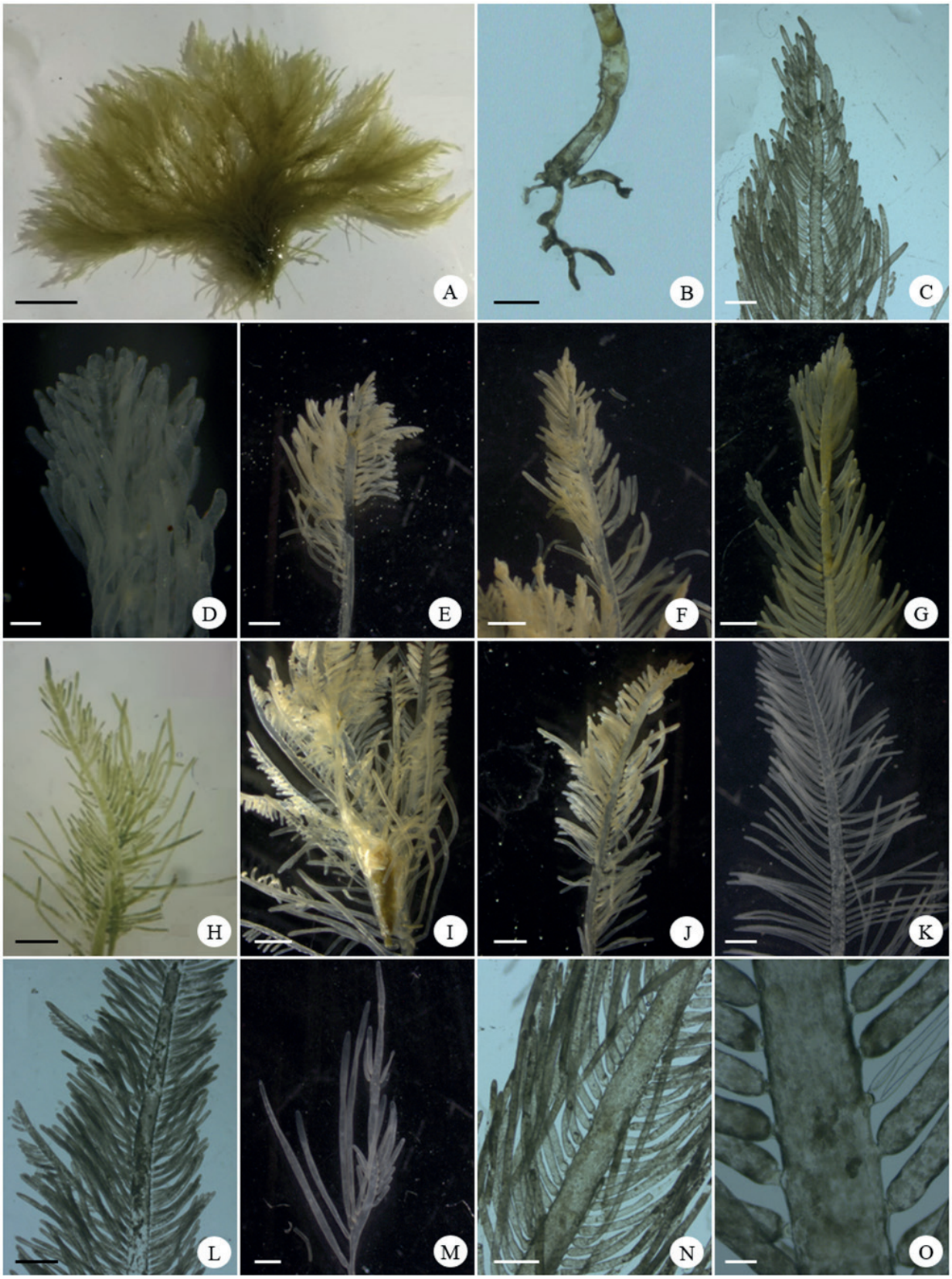

Figure 5. *Bryopsis pennata* (morphotype *Bryopsis* "*plumosa*"). **A.** Habit of the thallus. **B.** Detail of the basal portion showing rhizoids. **C-D.** Detail of the variation of the apices of the thallus. **E-M.** Detail of the variation of the distribution of the branchlets on the thallus. **N-O.** Detail of the distribution of the branchlets. Note branchlets constricted at the base. Bars: **A** = 1 cm, **B-D**, **N**, **O** = 200 μm, **E-M** = 1,000 μm.

B. corymbosa (Cormaci *et al.* 2014), although, according to Lee *et al.* (1991), intermediate characteristics are common, making it difficult to distinguish these species. In the present study, none of the specimens analyzed morphologically fit the description of *B. corymbosa* or were grouped with sequences of this species available in the databases. The sequences of *B. corymbosa* included in the molecular analyses are from the western Mediterranean (France and Spain), regions near the type locality (Livorno, Italy), and they were positioned phylogenetically distant from the Brazilian samples. Thus, the occurrence of *B. corymbosa* was not confirmed for the Pernambuco coast.

Our molecular and morphological results allowed us to register only the occurrence of *B. pennata* var. *pennata* and *B. pennata* var. *secunda*. Samples of *B. pennata* analyzed in this study proved to be extremely plastic, and the strictly morphological analyses led us to previously identify some of our specimens as *B. plumosa*. Table 4 compares the morphological characteristics of the studied material in regard to habit, distribution and length of branchlets.

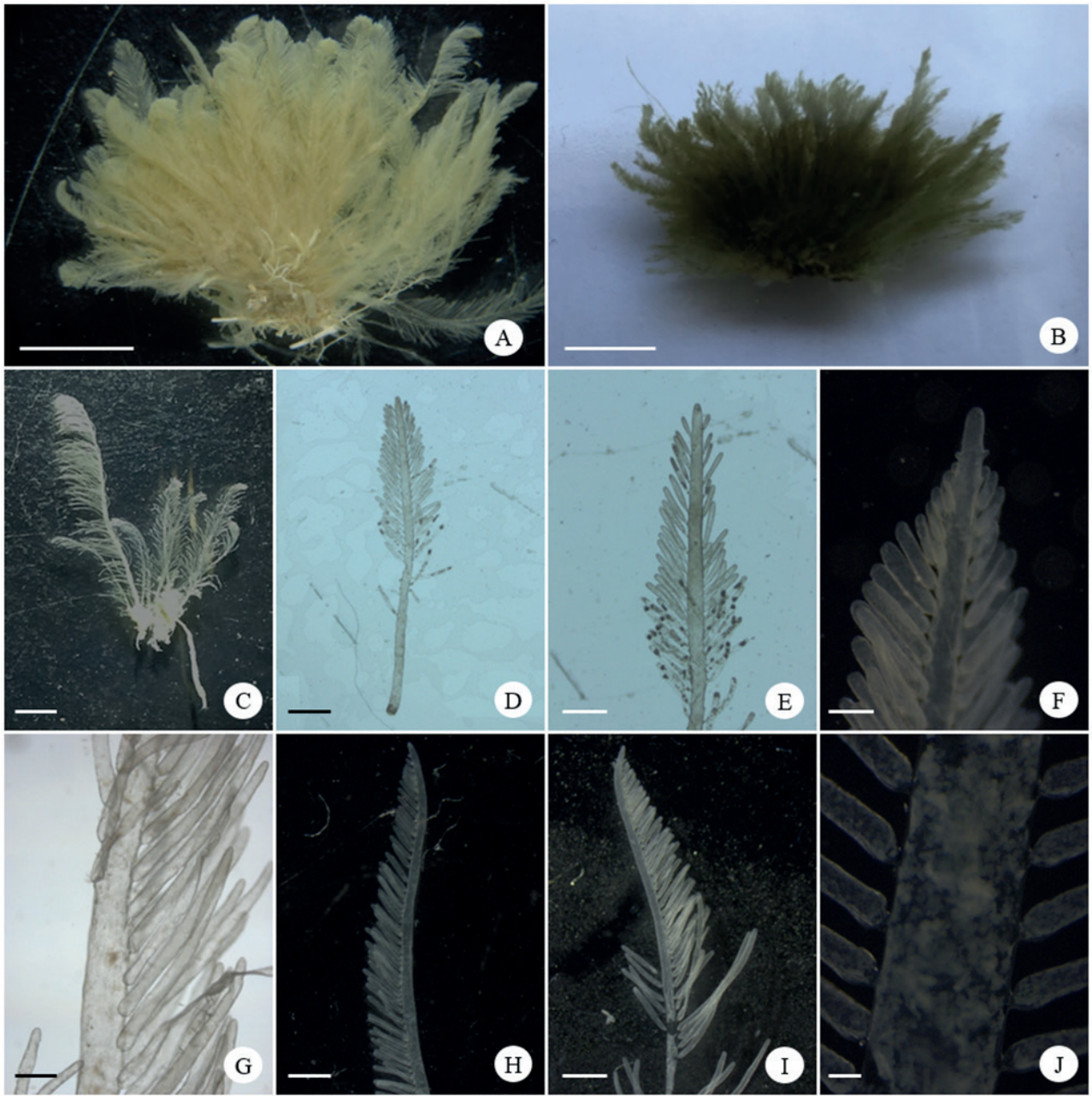

Figure 6. *Bryopsis pennata* var. *secunda*. **A-B.** Habit of the thalli. **C.** Part of a tuft showing distribution of the branchlets. Note axis with unilateral branchlets. **D.** Detail of an axis with opposite, distichous branchlets. **E-F.** Detail of the apical portions of the thallus with arrangement slightly alternate distichous of the branchlets. **G.** Detail of the branching pattern showing two rows of branchlets overlapping unilaterally. **H-I.** Detail of the thallus showing branchlets with mostly unilateral distribution. **J.** Detail of the distribution of the branchlets. Note branchlets constricted at the base. Bars: **A-B** = 1 cm, **C-D, I-J** = 1,000 µm, **E-H** = 200 µm.

Our analyses showed that there is an overlap in most of the compared characteristics; however, *B. pennata* specimens had an average thallus size smaller than that identified as *B.* "*plumosa*"; the latter also presented, in general, branches distributed discontinuously and longer, and often naked axes in its basal region. The diagnostic characteristics that separate *B. pennata* from *B. plumosa* are the habit and the size of the branchlets along the frond. In *B. pennata*, the thallus is lanceolate with branchlets uniformly sized, except near the apex, whereas in *B. plumosa* the thallus is pyramidal with branchlets non-uniformly sized, gradually smaller towards the apex (Krellwitz *et al.* 2001). In a study carried out in Praia de Serrambi (Pernambuco), Accioly (1989) observed that *B. pennata* occurred more frequently in the subtidal zone during the rainy season and *B. plumosa* in the intertidal zone during the dry season. However, our results showed that specimens of *B. pennata* and the *B.* "*plumosa*" co-occurred in some sampling sites (Cupe, Ilha de Santo Aleixo, Enseada dos Corais) at the same distribution range (intertidal) and essentially in dry season, and it was not possible to establish any distribution pattern, even in the intertidal zonation, or attribute the morphological variations observed to differences in environmental conditions. Although plants morphologically typical of *B. pennata* and *B. plumosa* could be recognized in the studied material, our molecular results for the two markers used, clearly refuted the occurrence of *B. plumosa* for the Pernambuco coast because B. *pennata-B.* "*plumosa*" were grouped with very low genetic divergence, 0-0.21 % for *tuf*A, and no divergence for *rbc*L. Our molecular analyses showed that *B. plumosa* is polyphyletic with a high genetic divergence (Tabs. 1, 3), corroborating the polyphyletism previously highlighted for this species by Ciancia *et al.* (2012). None of the Brazilian sequences previously identified as *B. plumosa* matched with samples of *B. plumosa* available in the databases, including sequences of the genetic species of *B. plumosa* from the French coast of the British Channel near the type locality (Devoshire, England) and whose divergence was high (12-13.6 % for *tuf*A and 6.7-8.4 % for *rbc*L), leaving no doubt that *B. plumosa* does not occur in the studied area. Thus, due to the phylogenetic positioning, the low genetic divergence observed between the *B. pennata* and "*B. plumosa*" and the identification of specimens morphologically typical of *B. pennata* in the analyzed material, we chose to cite our specimens under the name of *B. pennata*. Future comparisons with sequences from the type locality of *B. pennata* are necessary to confirm this taxon for the Pernambuco coast and Brazil.

Likewise, the sample from Argentina (JQ755423) with high genetic divergence from others *B. plumosa* (7.0 % to 7.2 % for *tuf*A and 2.3-2.6 % for *rbc*L, Tabs. 2, 3) was generated by Ciancia *et al.* (2012) having been described, illustrated and sequenced for *tuf*A and *rbc*L genes by these authors, confirming its identification and reinforcing to be *B. plumosa,* a complex of cryptic species. The recognition of cryptic and polymorphic species for *Bryopsis* has already been reported in previous molecular studies (Krellwitz *et al.* 2001; Lü & Wang 2011; Hollants *et al.* 2013; Tufiño-Velázquez & Pedroche 2019). Similar to that observed in our study in which *B. pennata* was shown to be polymorphic and included the morphotype *B.* "*plumosa*", Tufiño-Velázquez & Pedroche (2019), using the plastid *psb*B gene, concluded that *B. pennata* is the only species present in the Mexican Atlantic, and that the citations under the name of *B. hypnoides* and *B. plumosa* are names misapplied or uncertain, and even considered the possibility of *B. ramulosa* and *B. halliae* as being synonymous with *B. pennata*.

The analyzed specimens of *B. pennata* var. *secunda* showed diagnostic characteristics of this variety, differing from the typical variety by the small size of the frond (1.5 to 2.0 cm high in *B. pennata* var. *secunda* and 2.5 to 12 cm in *B. pennata* var. *pennata*), incurved apex, and unilateral distribution of branchlets (Tab. 4, Fig. 6C-I). Although there are no sequences of *B. pennata* var. *secunda* from the type locality (Key West, Florida, USA), available in the databases for comparison, we consider that the phylogenetic position and the genetic divergence observed between *B. pennata* var. *secunda* and the typical variety, mainly for the DNA barcode marker *tuf*A (0.96-1.57 %), support the maintenance of the taxonomic *status* of Brazilian samples as var. *secunda*, a decision also supported by the morphological data highlighted above. This is the first citation of *B. pennata* var. *secunda* for northeastern Brazil. This variety was previously cited only for the southeast (Espírito Santo state) and southern (Paraná, Santa Catarina states) regions of Brazil (INCT 2020).

There are no studies that report the intra- and interspecific divergences for the *tuf*A and *rbc*L markers for *Bryopsis*. However, the range of intraspecific divergence value for *tuf*A observed in this study for Brazilian samples (0-1.57 %) is relatively within the range of variation found for other Bryopsidalean genera, such as *Halimeda* (0-1.4 %) (Ximenes *et al.* 2019). The interspecific divergence observed for *tuf*A (3.3-16.2 %) is relatively within the range reported for *Halimeda*, 0.9-12.4 % by Ximenes *et al.* (2017) and 2.0-6.2 % by Ximenes *et al.* (2019), as well as for *Codium* (Bryopsidales), 1.44-14.65 %, and *Ulva* (Ulvales), 0.91-9.1 % reported by Saunders & Kucera (2010)

Intraspecific divergence within *Bryopsis* for *rbc*L (0-0.4 %) was lower than that observed for *Halimeda* (0.8-1.2 %) by Ximenes *et al.* (2019), whereas the range of interspecific variation for this marker (6.7-11.6 %) is within the range reported for *Halimeda*, 0.8-11.7 % by Ximenes *et al.* (2017) and 4.5-5.0 % by Ximenes *et al.* (2019). For the *psb*B marker, Tufiño-Velázquez & Pedroche (2019) proposed a genetic distance range between 5 % to 12 % for species discrimination and, therefore, considered a distance of up to 4.9 % as intraspecific, a higher value than that obtained for *tuf*A and *rbc*L in this study.

Our results from the study of the Brazilian material showed that *tuf*A was more efficient in delimiting species

(and infraspecific categories) than *rbc*L, confirming its use as a DNA barcode for green algae. The molecular data obtained so far for *Bryopsis* showed that a taxonomic revision for the genus is necessary, including a wide sampling on the Brazilian coast, in order to unveil the true diversity of the genus and clarify the taxonomic entities referenced for Brazil.

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References

Accioly MC. 1989. Clorofíceas marinhas bentônicas da praia de Serrambi (Município de Ipojuca-Estado de Pernambuco). Monograph, Universidade Federal Rural de Pernambuco, Recife.

Altschul SF, Madden TL, Schäffer AA, *et al*. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389-3402.

Angeiras JAP. 1995. Clorofíceas bentônicas do litoral sul do Estado de Pernambuco. MSc Thesis, Universidade Federal Rural de Pernambuco, Recife.

Barros NCG. 2013. Algas marinhas bentônicas como bioindicadoras da qualidade ambiental em área recifal de Tamandaré, Pernambuco, Brasil. PhD Thesis, Universidade Federal de Pernambuco, Recife.

Belton GS, Reine WFPH, Huisman JM, Draisma SGA, Gurgel CFD. 2014. Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa-peltata* complex (Chlorophyta, Caulerpaceae). Journal of Phycology 50: 32-54.

Burgos DC, Pereira SMB, Bandeira-Pedrosa ME. 2009. Levantamento florístico das Rodofíceas do Arquipélago de São Pedro e São Paulo (ASPSP) - Brasil. Acta Botanica Brasilica 23: 1110-1118.

Carvalho VF, Oliveira-Carvalho MF, Pedrosa ME, Pereira SMB. 2013. Benthic chlorophytes from a coastal island in the Oriental Northeast of Brazil. Brazilian Journal of Botany 36: 203-210.

Ciancia M, Alberghina J, Arata PX, *et al*. 2012. Characterization of cell wall polysaccharides of the coenocytic green seaweed *Bryopsis plumosa* (Bryopsidaceae, Chlorophyta) from the Argentine coast. Journal of Phycology 48: 326-335.

Collins FS, Hervey AB. 1917. The algae of Bermuda. Proceedings of the American Academy of Arts and Sciences 53: 1-195.

Coppejans E, Leliaert F, Verbruggen H, *et al*. 2004. The marine green and brown algae of Rodrigues (Mauritius, Indian Ocean). Journal of Natural History 38: 2959-3019.

Cormaci M, Furnari G, Alongi G. 2014. Flora marina bentonica del Mediterraneo: Chlorophyta. Bullettin of the Gioenia Academy of Natural Sciences of Catania 47: 11-436.

Cremen MCM, Leliaert F, West J, *et al*. 2019. Reassessment of the classification of Bryopsidales (Chlorophyta) based on chloroplast phylogenomic analyses. Molecular Phylogenetics and Evolution 130: 397-405.

Curtis N, Dawes C, Pierce S. 2008. Phylogenetic analysis of the large subunit rubisco gene supports the exclusion of *Arainvillea* and *Cladocephalus* from the Udoteaceae (Bryopsidales, Chlorophyta). Journal of Phycology 44: 761-767.

Cutrim MVJ. 1990. Distribuição das macroalgas na região entremarés do recife da Praia de Piedade, Município de Jaboatão dos Guararapes (Estado de Pernambuco-Brasil). MSc Thesis, Universidade Federal Rural de Pernambuco, Recife.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772-772.

Dijoux L, Verbruggen H, Mattio L, Duong N, Payri C. 2012. Diversity of *Halimeda* (Bryopsidales, Chlorophyta) in New Caledonia: a combined morphological and molecular study. Journal of Phycology 48: 1465–1481.

Famà P, Kooistra WCHF, Zuccarello G. 2002. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. Journal of Phycology 38: 1040-1050.

Freshwater DW, Fredericq S, Bradley SB, Hommersand MH, Chase MW. 1994. A gene phylogeny of the red algae (Rhodophyta) based on *rbc*L. Proceedings of National Academy of Sciences of the United States of America 91: 7281-7285.

Freshwater DW, Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. Phycologia 33: 187-194.

Gepp A, Gepp ES. 1908. Marine Algae (Chlorophyceae and Phaeophyceae) and Marine Phanerogams of the 'Sealark'Expedition, collected by J. Stanley Gardiner, MA, FRS, FLS. Transactions of the Linnean Society of London. 2nd. Series. Botany 7: 163-188.

Guimaraens MA, Oliveira-Carvalho MF, Aquino RE. 2015. Spatiotemporal variations of *Tricolia affinis* (CB Adams, 1850) associated with the macroalgal community on reef stretches at Piedade beach, southern coast of Pernambuco State, Brazil. African Journal of Plant Science 9: 196-204.

Guiry MD, Guiry GME. 2020. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. 25 Jan. 2020.

Hall JA, Fucíková K, Chien L, Lewis LA, Karol KG. 2010. An assessment of proposed DNA barcodes in freshwater green algae. Cryptogamie Algologie 31: 529-555.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. Vol. 41. London, Information Retrieval Ltd., c1979-c2000. p. 95-98.

Händeler K, Wägele H, Wahrmund U, Rüdinger M, Knoop V. 2010. Slugs' last meals: molecular identification of sequestered chloroplasts from different algal origins in *Sacoglossa* (Opisthobranchia, Gastropoda). Molecular Ecology Resources 10: 968-978.

Harvey WH. 1858. Nereis boreali-americana: or, contributions to a history of the marine algae Of North America. In: Harvey W H, Mria M. (eds.) Contributions to a history of the marine algae of North America. Part III. Vol. 10. Washington, Smithsonian Institution, Chlorospermeae Smithsonian Contributions to Knowledge. p. 1-140.

Holdt SL, Kraan S. 2011. Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology 23: 543-597.

Hollants J, Leliaert F, Verbruggen H, Willems A, Clerck O. 2013. Permanent residents or temporary lodgers: characterizing intracellular bacterial communities in the siphonous green alga *Bryopsis*. Proceedings of the Royal Society B: Biological Sciences 280: 20122659. doi: 10.1098/rspb.2012.2659

Horta PA, Amancio E, Coimbra CS, Oliveira EC. 2001. Considerações sobre a distribuição e origem da flora de macroalgas marinhas brasileiras. Hoehnea 28: 243-265.

Howe MA. 1928. Notes on some marine algae from Brazil and Barbados. Journal of the Washington Academy of Sciences 18: 186-194.

Ibrahim M, Salman M, Kamal S, Rehman S, Razzaq A, Akash SH. 2017. Algae-based biologically active compounds. In: Zia KM, Zuber M, Ali M. (eds.) Algae Based Polymers, Blends, and Composites. Amsterdam, Netherlands, Elsevier. p. 155-271.

INCT - Herbário Virtual da Flora e dos Fungos. 2020. http://inct.splink.org.br. 13 Jan. 2020.

Joly AB. 1957. Contribuição ao conhecimento da flora ficológica marinha da Baía de Santos e Arredores. Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo 14: 3-199.

Kapraun DF, Shipley MJ. 1990. Karyology and nuclear DNA quantification in *Bryopsis* (Chlorophyta) from North Carolina, USA. Phycologia 29: 443-453.

Kazi MA, Reddy CRK, Jha B. 2013. Molecular phylogeny and barcoding of *Caulerpa* (Bryopsidales) based on the *tuf*A, *rbc*L, 18S rDNA and ITS rDNA genes. PLOS ONE 8: e82438. doi: 10.1371/journal.pone.0082438

Krellwitz EC, Kowallik KV, Manos PS. 2001. Molecular and morphological analyses of *Bryopsis* (Bryopsidales, Chlorophyta) from the western North Atlantic and Caribbean. Phycologia 40: 330-339.

Lam DW, Zechman FW. 2006. Phylogenetic analyses of the Bryopsidales (Ulvophyceae, Chlorophyta) based on Rubisco large subunit gene sequences. Journal of Phycology 42: 669-678.

Lamouroux JVF. 1809. Observations sur la physiologie des algues marines, et description de cinq nouveau genres de cette famille. Nouveau Bulletin des Sciences, par la Société Philomathique de Paris 1 : 330-333.

Lee WJ, Boo SM, Lee K. 1991. Notes on the genus *Bryopsis* (Bryopsidaceae, Chlorophyta) from Ullungdo Island, Korea. Korean Journal of Phycology 6: 23-29.

Lefranc F, Koutsaviti A, Ioannou E *et al*. 2019. Algae metabolites: from in vitro growth inhibitory effects to promising anticancer activity. Natural Product Reports 36: 810-841.

Leliaert F, Lopez-Bautista JM. 2015. The chloroplast genomes of *Bryopsis plumosa* and *Tydemania expeditionis* (Bryopsidales, Chlorophyta): compact genomes and genes of bacterial origin. BioMed Central Genomics 16: 204. doi: 10.1186/s12864-015-1418-3

Leliaert F, Verbruggen H, Vanormelingen P, *et al*. 2014. DNA - based species delimitation in algae. European Journal of Phycology 49: 179-196.

Lü F, Wang G. 2011. Sequence and phylogenetic analyses of the chloroplast 16S rRNA, *tuf*A, and *rbc*L genes from *Bryopsis hypnoides*. Chinese Journal of Oceanology and Limnology 29: 922.

Luetzelburg P. 1922-23. Estudo Botânico do Nordeste. Vol. 1. Ministério de Viação e Obras Públicas. Rio de Janeiro, Inspetoria Federal de Obras contra as Secas.

Martius CFP. 1833. Algae, lichenes, hepaticae. In: Martius CFP, Eschweiler F, Nees AB, Esenbeck CG. (eds.) Flora brasiliensis seu enumeratio plantarum in Brasília tam sua sponte quam accendente cultura provenientium Vol. I. Stuttgart & Tuebingen Sumptibus J. G Cottae. p. 1-390.

McCourt RM, Karol KG, Bell J, *et al*. 2000. Phylogeny of the conjugating green algae (Zygnemophyceae) based on *rbc*L sequences. Journal of Phycology 36: 747-758l.

Miller KA, Aguilar-Rosas LE, Pedroche FF. 2011. A review of non-native seaweeds from California, USA and Baja California, Mexico. Hidrobiológica 21: 365-379.

Morabito M, Gargiulo G, Genovese G. 2010. A review of life history pathways in *Bryopsis*. Atti della Accademia Peloritana dei Pericolanti-Classe di Scienze Fisiche, Matematiche e Naturali. 88: C1A1002005. doi: 10.1478/C1471A1002005.

Muñoz AOM, Pereira SMB. 1997. Caracterização quali-quantitativa das comunidades de macroalgas nas formações recifais da Praia do Cupe-Pernambuco (Brasil). Trabalhos Oceanográficos da Universidade Federal de Pernambuco 25: 93-109.

Nguyen LT, Schmidt HA, Haeseler AV, Minh B. 2015. Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268-274.

Oliveira-Carvalho MDF, Oliveira MC, Pereira SMB, Verbruggen H. 2012. Phylogenetic analysis of *Codium* species from Brazil, with the description of the new species *C. pernambucensis* (Bryopsidales, Chlorophyta). European Journal of Phycology 47: 355-365.

Oliveira-Filho EC. 1977. Algas marinhas bentônicas do Brasil. PhD thesis, Universidade de São Paulo, São Paulo.

Pedrini A, Ugadim Y, Pereira SMB, Braga MR. 1992. Algas marinhas bentônicas do Arquipélago de Fernando Noronha, Brasil. Boletim de Botânica da Universidade de São Paulo 13: 93-101.

Pereira SMB, Accioly MC. 1998. Clorofíceas Marinhas Bentônicas da Praia de Serrambi, Pernambuco, Brasil. Acta Botanica Brasilica 12: 25-52.

Pereira SMB, Mansilla AOM, Cocentino ALM. 1996. Ecological aspects of a benthic marine algal community in Southeast Bay, Archipelago of Fernando de Noronha-Brazil. Trabalhos Oceanográficos da Universidade Federal de Pernambuco 24: 157-163.

Provan J, Murphy S, Maggs CA. 2004. Universal plastid primers for Chlorophyta and Rhodophyta. European Journal of Phycology 39: 43-50.

Ribeiro FA, Travassos A, Gestinari LM, *et al*. 2008. Análise quali-quantitativa das populações algáceas de um trecho recifal na Praia de Boa Viagem, PE. Oecologia Brasiliensis 12: 222-228.

Rietema H. 1975. Comparative investigations on the life-histories and reproduction of some species in the siphoneous green algal genera *Bryopsis* an *Derbesia*. PhD Thesis, University of Groningen, Netherlands.

Ronquist F, Teslenko M, Mark PVD, *et al*. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539-542.

Russell DJ. 2000. Identification manual for dietary vegetation of the Hawaiian green turtle *Chelonia mydas*. Florida, USA, US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center.

Santos AA, Cocentino ALM, Vasconcelos-Reis TN. 2006. Macroalgas como indicadoras da qualidade ambiental da Praia de Boa Viagem-Pernambuco, Brasil. Boletim Técnico-Científico do Centro de Pesquisa e Extensão Pesqueira do Nordeste 14: 25-33.

Saunders GW, Kucera H. 2010. An evaluation of *rbc*L, *tuf*A, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogamie Algologie 31: 487-528.

Schmidt OC. 1924. Meeresalgen der Sammlung von Lützelburg aus Brasilien. Hedwigia 65: 85-100.

Shimada S, Hiraoka M, Nabata S, Iima M, Masuda M. 2003. Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva*. Phycological Research 51: 99-108.

Silva EG. 2013. Estrutura e composição das comunidades de Amphipodas associadas às macroalgas dos recifes de arenito da Baía de Suape, PE-Brasil. MSc Thesis, Universidade Federal de Pernambuco, Recife.

Silva PC, Basson PW, Moe RL. 1996. Catalogue of the benthic marine algae of the Indian Ocean. Vol. 79. California, University of California Publications in Botany.

Silva RSVP. 2018. Carcinofauna associada ao fital *Caulerpa racemosa* (Forsskål) J. Agardh e *Bryopsis* spp. do Arquipélago de São Pedro e São Paulo - Brasil. Tropical Oceanography 46: 1-25.

Simões IP, Guimaraens MA, Carvalho MFO, Valdevino J, Pereira SMB. 2009. Avaliação florística e sucessão ecológica das macroalgas em recifes na Praia de Piedade (PE). Neotropical Biology and Conservation 4: 49-56.

Soares LP, Fujii MT. 2012. Novas ocorrências de macroalgas marinhas bentônicas no estado de Pernambuco, Brasil. Rodriguésia 63: 557-570.

Song W, Wang Z, Li Y, Han H, Zhang X. 2019. Tracking the original source of the green tides in the Bohai Sea, China. Estuarine, Coastal and Shelf Science 219: 354-362.

Sousa GS, Cocentino ALM. 2004. Macroalgas como indicadoras da qualidade ambiental da Praia de Piedade-PE. Tropical Oceanography 32: 1-22.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods), version, 4, b10. Sunderland, MA, USA, Sinauer Associates.

Széchy MTM, Nassar CAG, Falcão C, Maurat MCS. 1989. Contribuição ao inventário das algas marinhas bentônicas de Fernando de Noronha. Rodriguésia 41: 53-61.

Tufiño-Velázquez RC, Pedroche FF. 2019. Las especies del género *Bryopsis* (Chlorophyta) presentes en las costas del Atlántico mexicano. Revista Mexicana de Biodiversidad 90: e902679. doi: 10.22201/ib.20078706e.2019.90.2679

Verbruggen H, Ashworth M, LoDuca ST, *et al*. 2009. A multi-locus time-calibrated phylogeny of the siphonous green algae. Molecular Phylogenetics and Evolution 50: 642-653.

Villaça R, Pedrini AG, Pereira SMB, Figueiredo MDO. 2006. Flora marinha bentônica das ilhas oceânicas brasileiras. In: Alves RJV, Castro JWA. (eds.) Ilhas Oceânicas Brasileiras da Pesquisa ao Manejo. Brasília, Ministério do Meio Ambiente. p. 105-146.

Wade RM, Sherwood AR. 2017. Molecular determination of kleptoplast origins from the sea slug *Plakobranchus ocellatus* (Sacoglossa,

Gastropoda) reveals cryptic bryopsidalean (Chlorophyta) diversity in the Hawaiian Islands. Journal of Phycology 53: 467-475.

Williams SL, Smith JE. 2007. A global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annual Review of Ecology, Evolution, and Systematics 38: 327-359.

Wynne MJ. 2005. Two new species of *Bryopsis* (Ulvophyceae, Chlorophyta) from the Sultanate of Oman, with a census of currently recognized species in the genus. Contributions from the University of Michigan Herbarium 24: 229-256.

Wynne MJ. 2017. A checklist of benthic marine algae of the tropical and subtropical western Atlantic: fourth revision. Nova Hedwigia, Beihefte 145: 1-202. ISBN 978-3-443-51067-1

Ximenes CF, Cassano V, Oliveira-Carvalho MDF, *et al*. 2017. Systematics of the genus *Halimeda* (Bryopsidales, Chlorophyta) in Brazil including the description of *Halimeda jolyana* sp. nov. Phycologia 56: 369-381.

Ximenes CF, Oliveira-Carvalho MDF, Bandeira-Pedrosa ME, Cassano V. 2019. Updates on Section *Pseudo-opuntia* of *Halimeda*: phylogenetic analyses of *H. soniae* sp. nov. (Bryopsidales, Chlorophyta) along the Brazilian coast. Botanica Marina 62: 327-336.

Yu KX, Wong CL, Ahmad R, Jantan I. 2015. Mosquitocidal and oviposition repellent activities of the extracts of seaweed *Bryopsis pennata* on *Aedes aegypti* and *Aedes albopictus*. Molecules 20: 14082-14102.

Zeller G. 1876. Algae brasiliensis circa Rio de Janeiro a Dr. Glaziou, Horti publici directore collectae. In: Warming E. (ed.) Symbolae ad floram Brasiliae centralis cognoscendam. Vol. 22. København, Videnskabernes Meddelelser Natur Foren. p. 426-432.

Zhang Z, Wang F, Wang X, Liu X, Hou Y, Zhang Q. 2010. Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. Carbohydrate Polymers 82: 118-121.